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Note

The Effects of Germination on Chemical Composition of Peanut Seed

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With regard to important changes in chemical composition, germination can be considered a valuable processing technique for modifying nutrient components of legume seeds. In this study, the changes in major chemical composition of peanut seeds were evaluated during short-term germination. The contents of water, minerals, aspartic acid, methionine, proline, folic acid, thiamine and total phenolics increased dramatically in peanut cotyledons and sprouts after germination, while the fat, riboflavin and ascorbic acid contents decreased markedly. The content of total amino acids, moreover, showed no obvious decrease, and the relative amount of some limiting and essential amino acids clearly increased after germination in peanut seed. This suggests that sprouts produced from peanut seeds could serve as a healthy food containing low fat, high levels of minerals and flavonoids.

Keywords: peanut, seed, germination, chemical composition, protein, immunoreactivity

Introduction

Peanut (*Arachis hypogaea* L.) seeds are a good source of protein (16 − 36% protein), lipids (36 − 54% oil) (Knauft and Ozias-Akins, 1995), minerals [calcium (Ca) and magnesium (Mg)] and vitamins (Savage and Keenan, 1994). Peanut seeds are a major source of vegetable oil and other products, such as soup thickener where they double as nutritional enhancers; and snacks when cooked, roasted, dried or fried. In recent years, peanut sprouts as a new healthy vegetable have been available in some supermarkets in China, and many reports on their production process have appeared.

Various sprouted beans, especially mung beans, lentils and edible soybeans have been well known throughout the centuries in oriental culture and have also gained popularity in western countries. Many studies indicated higher levels of nutrients in germinated legumes in comparison with ungerminated originals. A marked increase of essential amino acid contents were observed in *Glycine* and *Phaseolus* beans (Lee and Karunanithy, 1990). The germination process, moreover, significantly increased ascorbic acid contents in mung bean, chickpea and cowpea (Bains *et al*., 2011), and led to a significant increase in riboflavin and total niacin contents in faba bean (Prodanov *et al*., 1997). Germinated leguminous seeds also showed increased contents of iron (Fe) and zinc (Khalil and Mansour, 1995; Bains *et al*., 2011). However, there is almost no information concerning the effects of germination on nutrient quality of peanut seeds.

The purpose of this study was to examine the effects of germination on nutritional properties of peanut seeds in order to provide some scientific basis for comprehensive utilization of peanut sprouts.

Materials and Methods

Seed and germination Dry peanut seeds were purchased from a local supermarket in the city of Wuxi in Jiangsu Province, China. The seeds were first soaked in boiled water for 10 min at 50℃, and then for 12 h at 20℃. Imbibed seeds were germinated for up to 5 d in a dark chamber regulated to 100% relative humidity and a steady temperature of 25℃. The seeds were rinsed every 12 h with boiled water at 25℃ during germination. Cotyledons and sprouts were weighed, packed and frozen in liquid nitrogen and kept at −80℃ for analysis.

Water content Fresh and dry (raw peanut seed) samples (n = 3)

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of 2.0 g were cut into small pieces of 2 mm, then dried in an oven at 100 − 105℃ (±1℃) to constant weight (GB 5009.3-2010).

Total soluble sugars (TSS) Samples of 0.2 g of dry peanut seed (raw material, RM) or 1.0 g of fresh germinated cotyledons and sprouts were crushed and then dissolved in 100 mL of distilled water. The filtrates were collected and stored at 4℃ for TSS determination. In 20 × 150 mm tubes, 5 mL of sample, standard (5, 10, 15, 20, 25, 30, 35 and 40 mg/L glucose standard solution) or water blank solution, was reacted for exactly 10 min in a boiling water bath with 10 mL of 0.2% anthrone reagent (prepared by dissolving 0.2 g of anthrone and 1.0 g of thiourea in 100 mL of reagent grade concentrated sulfuric acid). After cooling, absorbance at 625 nm was determined in a UV-visible spectrophotometer. TSS content was calculated as the weight of glucose per 100 g of sample dry weight.

Fat content The fat content of samples (n = 3) was determined in triplicate by comparing the weight of approximately 2 g of fresh (germinated cotyledons and sprouts) and dry (raw peanut seed) peanut seed tissues before and after fat removal by Soxhlet extraction with n-hexane (Kang *et al*., 2003).

Amino acid analysis Dry (60 mg) or fresh samples (100 − 600 mg) were hydrolyzed by heating for 24 h at 110℃ in 6 mol/L HCl under vacuum in sealed ampoules. The amino acid compositions of the acid hydrolysates were determined using amino-dedicated high-performance liquid chromatography, Agilent 1100 (Lee and Karunanithy, 1990).

Mineral content Determination of mineral contents was carried out using an atomic absorption spectrophotometer (Varian Spectr AA 220 Z, UK), fitted with appropriate monoelement hollow cathode lamps.

Powdered dry (1.0 g) and fresh samples (2 − 4 g) were incubated with concentrated HNO_3 (AR grade) overnight at room temperature, and then gently boiled for 4 h, cooled and made up to 50 mL with double distilled water. The solution was directly aspirated into an oxyacetylene flame (Lee and Karunanithy, 1990).

Water-soluble vitamins Ascorbic acid (vitamin C), thiamine (vitamin B_1), riboflavin (vitamin B_2), nicotinamide (vitamin B_3) and folic acid (vitamin B_{11}) were simultaneously determined according to the methods of Albalá-Hurtado *et al*. (1997) with some modifications. Standards of vitamins were purchased from Sigma. (1) Stock solution: 500 mg/L of folic acid in 5% (w/v) sodium bicarbonate; 100 mg/L riboflavin in 2.4% (v/v) aqueous acetic acid; 1000 mg/L of thiamine, nicotinamide and ascorbic acid in 2.4% (v/v) aqueous acetic acid. (2) Work solutions [all in 2.4% (v/v) aqueous acetic acid]: 0.01, 0.02, 0.1, 0.2, 0.3, 0.6 and 1.0 mg/L for thiamine, riboflavin and folic acid; 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/L for ascorbic acid; and 0.3, 0.5, 0.7, 0.8, 1, 2 and 2.5 mg/L for nicotinamide.

Of dry or fresh samples, 5.0 g was crushed and dissolved in 50 mL of 10% trichloroacetic acid. The mixture was ultrasonically extracted for 10 min and centrifuged for 10 min at 3000 *g*. Standard and sample solutions were protected from light and filtered through a 0.45-μm membrane before storage at 4℃.

Tannin content Tannin content was determined by the Folin-Ciocalteu method (Lin and Tan, 2007; Maisont and Narkrugsa, 2010), with some modifications. Briefly, 1.0-g dry or fresh samples were crushed and dissolved in 50 mL of distilled water. Then the mixtures were extracted in a boiling water bath (95 − 100℃) for 30 min and centrifuged for 4 min at 1250 *g*. Of supernatant, 1.0 mL was mixed with 5.0 mL of deionized water, 3 mL of 7.5% sodium carbonate (Na_2CO_3) and 1 mL of 10% Folin-Ciocalteu reagent. The absorbance of the reaction mixture was measured at 765 nm after incubation at room temperature for 2 h. Gallic acid was chosen as a standard.

Flavonoid content The content of total flavonoid was measured using a colorimetric assay (Zhang *et al*., 2010). Briefly, extracts (2.0-g samples in 10 mL of 70% methanol) of 2.5 mL were mixed with 0.15 mL of 5 g/100 g solution of sodium nitrite, and then 0.15 mL of 10 g/100 g solution of aluminum nitrate was added after shaking and incubation for 5 min. Next, 1 mL of 1 mol/L sodium hydrate solution was added after shaking and incubation for 6 min. The final mixture was shaken well and 70% methanol was added to make up 5 mL, and then the absorbance of these mixtures was measured at 500 nm. Rutin (Sigma) was used as a standard.

Results and Discussion

Water content Germination commences with the uptake of water by the dry seed (Derek Bewley, 1997).The water content of peanut seeds increased rapidly during soaking (0 d), and then the content increased only gradually (Fig. 1). The rapid initial uptake caused by imbibition is necessary for the initial resumption of metabolic activity, such as mitochondria and DNA repaired, and proteins synthesized (Derek Bewley, 1997). After 1, 3 and 5 d of germination, water content in sprouts reached 64.5, 88.6 and 90.7%, respectively, and were higher than the corresponding values in cotyledons about 59, 92 and 73%. The repair of membranes and organelles, metabolic activities and elongating of embryonic axes could account for the following gradual increases of water content in cotyledons and sprouts (Derek Bewley, 1997).

TSS and Fat content Germination produced marked changes in sugar (Fig. 2A) and fat (Fig. 2B) content. The TSS in cotyledons decreased rapidly after 1 d germination, and continued to decline, dropping by 25% at 3 d of ermination and by 50% at 5 d. In contrast, the contents in sprouts increased continuously with germination time, from 8.9 to 25.7 g/100 g at 1 d of germination compared to 5 d (Fig. 2A). A steady decrease in fat content of peanut cotyledons and sprouts could be observed during germination, from 41.2 and 37.6 g/100 g in cotyledons and sprouts at 1 d of germination, respectively, to corresponding values of 23.4 and 10.8 g/100 g at 5 d - decreases of about two- and three-fold (Fig. 2B). Peanut is a typical oil-storing seed, and sugars and fats

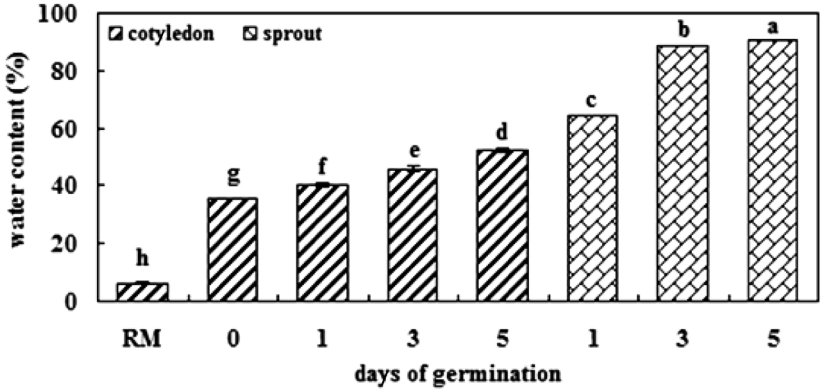

Fig. 1. Water content of raw and germinated peanut seeds. RM – raw peanut material. Bars with different letters represent values that are significantly different ($p < 0.05$); vertical bars indicate ± SD.

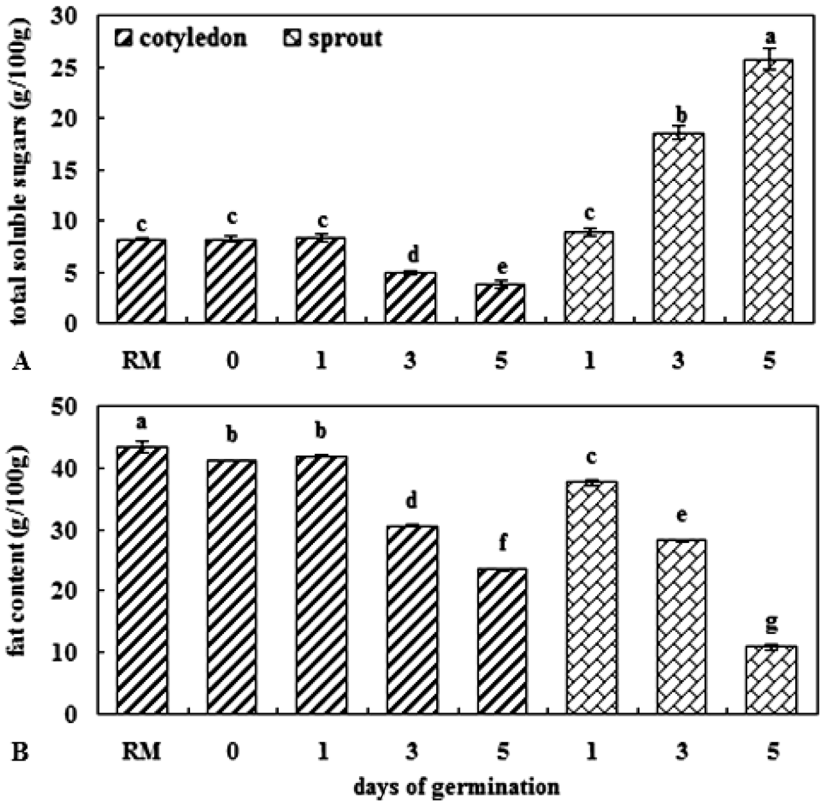

Fig. 2. Total soluble sugars (A) and fat content (B) of raw and germinated peanut seeds. RM – raw peanut material. Bars with different letters represent values that are significantly different ($p < 0.05$); vertical bars indicate ± SD.

are the major source of energy during its germination (Kornberg and Beevers, 1957). Thus, the significant decrease in sugar and fat contents of seeds during germination (Fig. 2) can be attributed to production of energy required for metabolic activity (Khalil, 1995), such as synthesis of RNA, DNA, structural proteins, enzymes and other biological molecules. Similar observations were reported by Bau *et al*. (1997) in soybean and Kumaraguru and Rajkumar (2011) in field pea, chick pea, black gram and lentil. However, the content of sugars in peanut (Fig. 2A) and other plant seed sprouts (Benítez *et al*., 2013) increased obviously during germination. Benítez *et al*. (2013) suggested that the increase of total sugar content during seed germination was mainly due to the rise in cellulosic glucose from metabolic reaction. Increases in cellulose have also been reported in germinated legume seeds (Trugo *et al*., 2000; Martín-Cabrejas *et al*., 2003).

Total amino acid and amino acid profile. There was a decline of 7.4% in amino acid content after soaking, but no marked change during germination (Fig. 3A). It is well known that peanut seeds are good source of protein. The amino acid contents in peanut cotyledons and sprouts remained constant (about 30%) during germination (Fig. 3A), indicating that the germination process did not markedly reduce the food protein quantity of peanut seed.

Similar results were obtained by Hahm *et al*. (2009) in sesame seeds. McOsker (1961) showed that lysine, methionine and threonine were limiting amino acids in peanut seeds. Notably, there was a marked increase in the relative amount of methionine in peanut sprouts in the present study (Fig. 3B). The relative amount of proline, an essential amino acid, clearly increased in germinated peanut cotyledons and sprouts (Fig. 3B). These increases may have positive affected nutritive value of peanut seeds.

Mineral contents Fe, Ca and Mg contents in germinated peanut sprouts were notably higher than that in cotyledons (Table 1). At 3 d of germination, the contents of Fe, Ca and Mg in cotyledons were about 27, 70 and 66% of that in sprouts, respectively. The changes in Fe, Ca and Mg contents during germination differed for cotyledons and sprouts. Fe and Mg contents in cotyledons showed no marked changes during germination, but Ca content increased following 1 d of germination. Fe and Ca contents steadily increased (4.85 − 5.08 and 170.91 − 222.30 mg/100 g, respectively) and Mg content slightly decreased (349.13 − 304.72 mg/100 g) in sprouts during germination. The contents of Fe, Ca and Mg in germinated peanut were noticeably higher than that in raw peanut (Table 1) – similar results were reported by Zieliński *et al*. (2006). However, Bains *et al*. (2011) reported no significant variation in Fe and Ca contents of mung bean and cowpea after different germination periods. Hahm *et al*. (2009) found that Ca content in sesame seeds increased after germination, and Mg content fluctuated slightly, whereas Fe content decreased. Khalil and Mansour (1995) also reported a decreased Mg content and an increased Fe content in germinated faba bean. The differences in mineral contents among different seeds may be due to differences in species or in the germination process: cooled, boiled water was used by Zieliński *et al*. (2006), similar to the present study; however, other studies rinsed seeds using distilled water. Various minerals are present in boiled water, but little or none in distilled water.

Water-soluble vitamins The changes in contents of ascorbic acid and B-group vitamins were erratic during peanut seed germination. The contents of ascorbic acid and riboflavin in cotyledons and sprouts dropped markedly (Fig. 4), ascorbic acid contents in cotyledons and sprouts at 5 d of germination decreased by about 20 and 50%, respectively, compared with samples at 1 d (Fig. 4A); Similarly, riboflavin contents decreased by about 70% in cotyledons; and was not detected in sprouts (Fig. 4B). However, thiamine, and especially folic acid, content rose during germination. The contents of folic acid in cotyledons and sprouts at the end of germination increased 1.4- and 2.5-fold compared with samples at 1 d of germination (Fig. 4B). Besides, the contents of nicotinamide showed no obvious changes both in cotyledons and sprouts during the entire germination period. To the best of our knowledge, there is little information in the literature concerning the effect of germination on contents of ascorbic acid, thiamine,

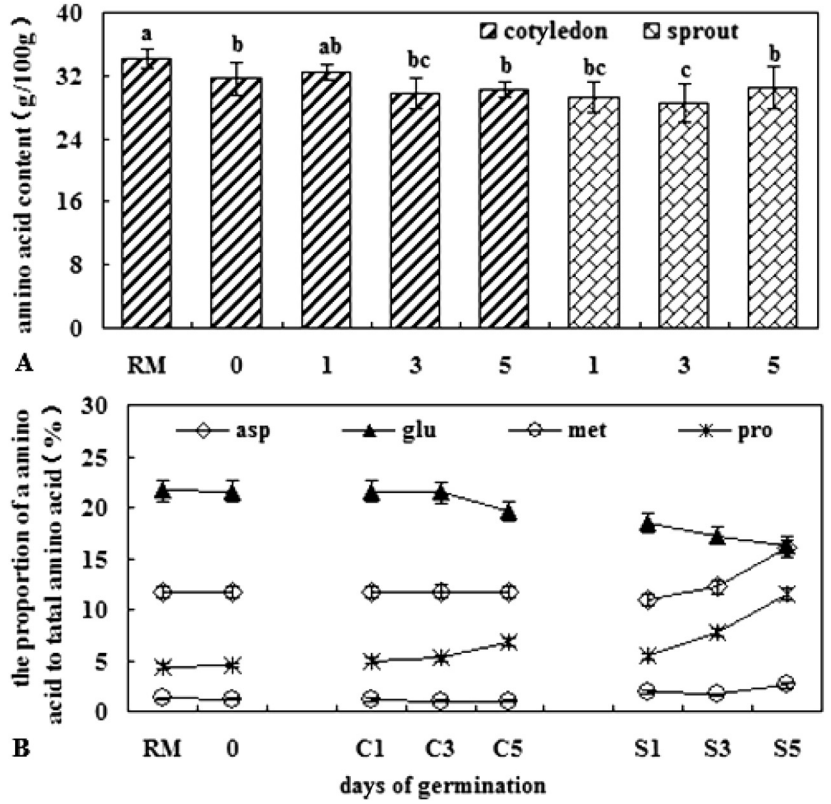

Fig. 3. Total amino acid (A) and amino acid relative amounts (B) of raw and germinated peanut seeds. RM – raw peanut material; C – cotyledons; S – sprouts. Bars with different letters represent values that are significantly different ($p < 0.05$); vertical bars indicate ± SD.

Table 1. Mineral contents (mg/100 g) of raw and germinated peanut seeds (RM – raw peanut material; C – cotyledons; S – sprouts).

Mineral	Days of germination							
	RM	0	C1	C3	C5	S1	S3	S5
Fe	1.31 ± 0.11	1.33 ± 0.23	1.22 ± 0.18	1.37 ± 0.31	1.24 ± 0.47	4.85 ± 0.50	4.67 ± 0.34	5.08 ± 0.52
Ca	72.70 ± 8.02	76.92 ± 13.22	98.21 ± 15.04	126.94 ± 12.78	124.92 ± 8.09	170.91 ± 14.42	182.26 ± 15.08	222.30 ± 19.04
Mg	201.61 ± 12.47	200.61 ± 14.85	234.90 ± 17.43	217.68 ± 13.75	232.06 ± 12.22	349.13 ± 17.04	328.96 ± 20.38	304.72 ± 15.76

Data are averages ± SD of three determinations.

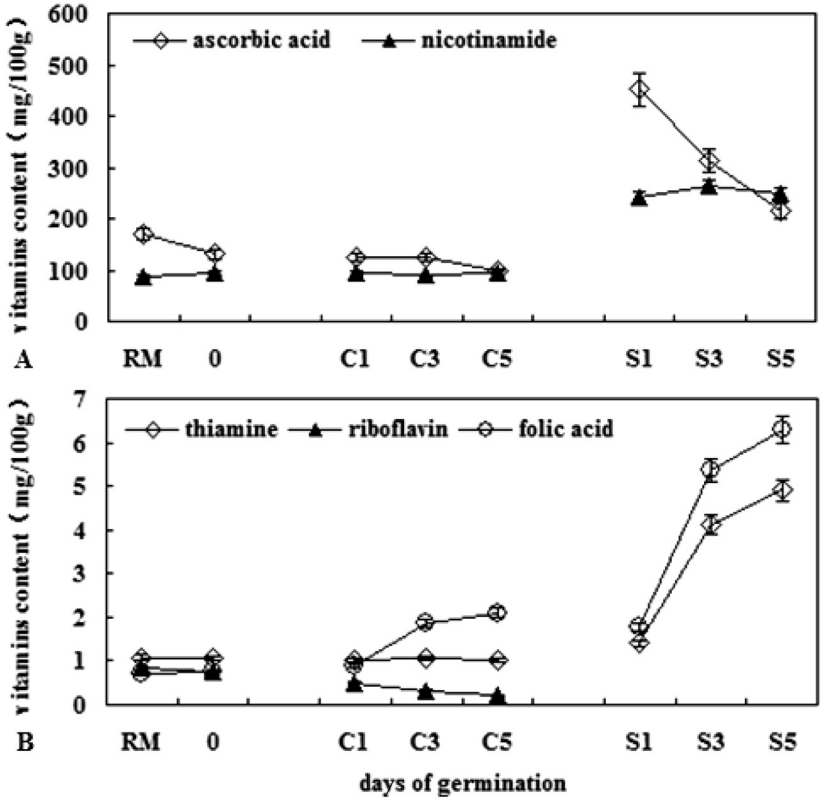

Fig. 4. Ascorbic acid (A), nicotinamide (A), thiamine (B), riboflavin (B) and folic acid (B) contents of raw and germinated peanut seeds. RM – raw peanut material; C – cotyledons; S – sprouts. Vertical bars indicate ± SD.

and other B-group vitamins in seeds, and no information relating to peanut seed. The results from other researchers concerning vitamin content changes in legumes during germination are also inconsistent. Bains *et al*. (2011) reported that germination significantly increased ascorbic acid in mung bean, chickpea and cowpea; however, Zieliński *et al*. (2006) showed an almost linear reduction in thiamine content during rapeseed germination, and a gradual increase of riboflavin content in sprouts throughout the germination. Prodanov *et al*. (1997) suggested that the conditions -such as the number of rinses, light levels and the time for seed germination - affected the contents of vitamins. The poor solubility in water and sensitivity to light of riboflavin could account for its low level in germinated peanut seed samples.

Tannin and flavonoid contents Tannins are astringent, bitter plant polyphenolic compounds, and were traditionally considered antinutritional because they can bind to and precipitate proteins and other organic compounds. However, flavonoids, a major class of tannins, have in recent years come to be viewed in a positive light by both scientists and consumers because of their antioxidant activity and anticancer effects. There was a general increase in tannin and flavonoid contents in germinated peanut seeds in the present study (Fig. 5). Tannin contents in cotyledons and sprouts reached their peaks of 17.53 and 44.87 g/kg at 5 and 3 d of germination, respectively, which were 13.6 and 70.7% higher than that at 1 d. There was a similar change trend for flavonoid contents in peanut seeds during germination: with 45 and 128% increases in cotyledons and sprouts after 5 d of germination compared to initial germination (0 d). Our findings are consistent with those of López-

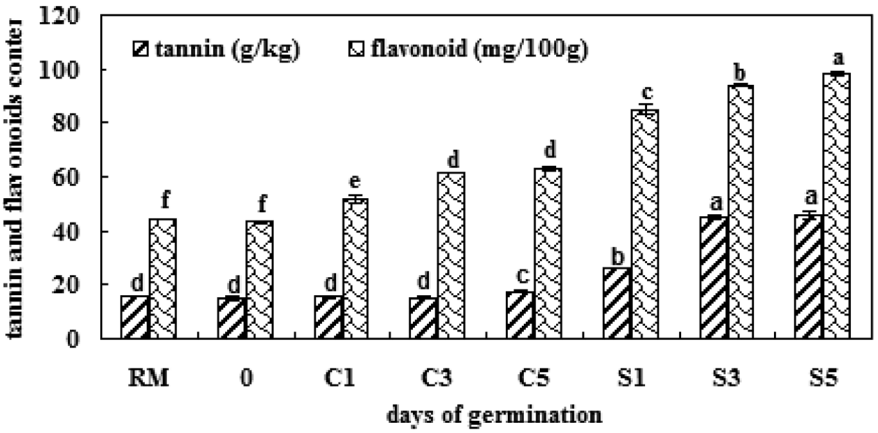

Fig. 5. Tannin and flavonoid content of raw and germinated peanut seeds. RM – raw peanut material; C – cotyledons; S – sprouts. Bars with different letters represent values that are significantly different ($p < 0.05$); vertical bars indicate ± SD.

Amorós *et al*. (2006) and Gharachorloo *et al*. (2012), which indicated that germination modified the quantity and quality of phenolic compounds of legumes that underwent a significant increase in antioxidant activity. Therefore, peanut sprouts could be used as a source of natural antioxidants.

Conclusions

The germination process obviously affected chemical composition of peanut seeds. The contents of minerals, aspartic acid, methionine, proline, folic acid, thiamine and total phenolics increased dramatically during germination, especially in sprouts. However, the contents of fat, riboflavin and ascorbic acid decreased markedly in germinated peanut cotyledons and sprouts. The germination conditions (rinse, light and time), moreover, affected the chemical composition of peanut seeds. A study of factors of germination process therefore will be needed to find optimal operation conditions.

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