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Germination of Peanut Kernels to Enhance Resveratrol Biosynthesis and Prepare Sprouts as a Functional Vegetable

KUO-HSI WANG,† YU-HSUAN LAI,‡ JU-CHUN CHANG,‡ TIN-FU KO,† SHYI-LIANG SHYU,† AND ROBIN Y.-Y. CHIOU*,‡

Graduate Institute of Food Science, and Graduate Institute of Biotechnology, National Chiayi University, Chiayi, Taiwan

Bioactive benefits of resveratrol in the diets have attracted extensive interests of the public. Peanut is one of the potent natural sources of resveratrol. In this study, germination of peanut kernels to enhance resveratrol biosynthesis and preparation of sprouts as a functional vegetable was conducted. When the rehydrated kernels of three peanut cultivars were germinated at 25 °C and relative humidity 95% in dark for 9 days, resveratrol contents increased significantly from the range of 2.3 to 4.5 μg/g up to the range of 11.7 to 25.7 μg/g depending upon peanut cultivar. In comparison with the sprout components, resveratrol contents were highest in the cotyledons, slightly lower in the roots, and not detected in the stems. When the sprouts were heated in boiling water for 2 min, resveratrol contents varied in a limited range. Methanol extracts of the freeze-dried sprouts exhibited potent 1,1-diphenyl-2-picryl-hydrazyl scavenging activity and antioxidative potency against linoleic acid oxidation. These activities increased with an increase of germination time. After 9 days of germination, total free amino acid, sucrose, and glucose contents increased significantly while crude protein contents decreased and the large sodium dodecyl sulfate polyacrylamide gel electrophoresis protein molecules of the kernels were extensively degraded. From a practical viewpoint, it is of potency to prepare peanut sprouts as a functional vegetable.

KEYWORDS: Peanut; sprout; resveratrol; antioxidative activity; SDS−PAGE; functional vegetable

INTRODUCTION

Resveratrol (3,4′,5-trihydroxystilbene), one of the inducible phytoalexins, has been intensively investigated and ranked as a potent bioactive phytochemical in the chemoprevention of cardiovascular diseases and cancer (*1*−*6*). These data indicate that the consumption of foods containing resveratrol to benefit disease prevention and even extension of lifespan (*7*) is likely. Resveratrol has been detected in the peanut kernels, roots, leaves, pods, and peanut-related foods (*8*−*13*). Chung et al. (*12*) have detected resveratrol synthesis mRNA in the peanut leaves, roots, pods, kernels, and kernel skins. These indicate that peanut is an available source of resveratrol. On the basis of the fact that the resveratrol content of peanut kernels is comparatively low (*8*, *9*) and the phytoalexin content of peanut kernels increases during germination and is enhanced by microbial infection and wounding (*14*, *15*), enhancement of resveratrol biosynthesis by subjecting peanut kernels to germination is expected.

Germination of plant seeds is a crucial step in the generation of a new offspring. During germination, a complex and sophisticated set of biochemical and physiological reactions progresses under a precisely controlled process. Isoflavone and flavonoid contents and general nutritional contributors increase during soybean germination (*16*−*18*). When black soybean, soybean, and lupin seeds were germinated for 48 h and applied as an ingredient in the rat diet, positive physiological effects were observed in the germinated beans (*19*). In this study, preparation of peanut sprouts with an attempt to enhance resveratrol biosynthesis was conducted. Sound and mature kernels of three commonly grown peanut cultivars were subjected to germination and resveratrol analysis. Antioxidative activities of the sprouts and compositional changes of the kernels before and after germination were investigated.

MATERIALS AND METHODS

Germination of Peanut Kernels and Sprout Preparation. Sound and mature peanut kernels of Tainan Selected 9 (TNS 9), Tainan 11 (TN 11), and Tainan 14 (TN 14) stored at −30 °C were used in this study. For each batch, after the stored kernels were tempered in bags at ambient temperature (24−26 °C) overnight, 100 visibly sound kernels were sorted, weighed, and rehydrated with tap water for 4 h at the ambient temperature. The kernels were placed on a plastic net tray and germinated under dark in a growth chamber (Hipoint 747FH, Hipoint Co., Kaohsiung, Taiwan) at 25 °C and 95% relative humidity for 9 days. After 3 days of incubation, the ungerminated kernels (without emergence of germ tube) were discarded to eliminate molding. After 9 days, the numbers and weights of sprouts were counted and weighed,

* To whom correspondence should be addressed. Tel: +8865-2717613. Fax: +8865-2775524. E-mail: rychiou@mail.ncyu.edu.tw.
† Graduate Institute of Food Science.
‡ Graduate Institute of Biotechnology.

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respectively. Sprout yield was estimated by dividing the sprout weight by that of the initial weight of 100 kernels destined for germination.

Sample Pretreatment. For each batch, 100 visibly sound kernels were subjected to germination as that described above. After 0, 3, 6, and 9 days of germination, a tray from each peanut cultivar was harvested and divided evenly into three sublots. From each sublot, six kernels or sprouts were randomly sampled, weighed, and followed by lyophilization (Lyvotac GT2, Finn-Aqua, Heraus, Germany). Also from each sublot, six sprouts were sampled, cut, and divided into three components including cotyledons, roots, and stems. The separated sprout components were weighed and subjected to lyophilization. After lyophilization and weight determination, the dried materials were pulverized with a cyclone mill to prepare whole sprout and sprout component powders. The powders were sealed in polyethylene plastic bags and stored under −30 °C for resveratrol and compositional analyses.

Determination of Resveratrol Content and Resveratrol Stability of Peanut Sprouts when Subjected to Heating. Resveratrol was extracted from the freeze-dried sprout and component powders following the procedure reported by Chen et al. (*11*). A high-performance liquid chromatography (HPLC) pump in connection with an UV detector (L-7100 pump and L-7420 detector, Hitachi Co. Ltd., Tokyo, Japan) monitored at 307 nm, and a reverse phase thermal hypersil column (ODS, 250 mm × 4.6 mm, 5 μm, Thermo Hypersil Ltd., Cheshire, England) was employed with a gradient solvent system passing through a degasser (LDG-2410, Uniflows Co., Tokyo, Japan). The solvent was initiated from 30 to 90% of aqueous methanol solution in 16 min and held for an additional 2 min. The flow rate and injection volume were 1 mL/min and 20 μL, respectively. A standard resveratrol (Sigma Chemical Co., St. Louis, MO) was run under an identical condition as a reference for quantification. For monitoring resveratrol stability, a series of three peanut sprouts (after 9 days of germination) were cooked in a boiling water bath for 0, 0.5, 1.0, and 2 min, respectively. After they were heated, peanut sprouts were cooled with tap water and subjected to lyophilization to prepare dried sprout powders. From the powders, resveratrol contents were determined as described above.

Preparation of Methanol Extracts and Subsequent Determination of Antioxidative Activities. For extraction of the antioxidants, 0.1 g of freeze-dried whole sprout or sprout component powder was deposited into a 10 mL centrifuge tube (Nalgene 3114-0010, Nalge Co., Rochester, NY). The powder was homogenized with 4 mL of 80% (v/v) methanol for 1 min at 15 000 rpm using a polytron homogenizer equipped with a small aggregate probe (Polytron PT3000; Kinematica AG, Littaw, Switzland). The probe was rinsed with an additional 1 mL of 80% methanol and pooled. The tubes were screw-capped and heated for 30 min in a water bath at 70 °C with occasional shaking. Then, the tubes were centrifuged at 19000*g* at 20 °C for 15 min and the supernatants designated as methanol extracts were membrane filtered (0.2 μM) and stored at −30 °C for further analyses.

For determination of free radical scavenging activity of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), the method reported by Shimada et al. (*20*) was followed. DPPH solution (2 mM in *n*-butanol) as a free radical source was freshly prepared. A series of diluted solutions of the methanol extracts were prepared by diluting 0, 50, 100, and 200 μL of the methanol extracts with 80% methanol (v/v) to 1.0 mL of final volume. Solutions of 100 ppm of BHT (butylhydroxyl toluene) and vitamin E (α-tocopherol) (dissolved in methanol) were prepared as referenced counterparts. From each of the final solutions, 1.0 mL was deposited into a test tube, replenished with 0.1 mL of DPPH solution, incubated at 25 ± 1 °C for 30 min, and followed by absorbance determination at 517 nm. As a blank control, 1.0 mL of methanol was mixed with 0.1 mL of DPPH solution and incubated concurrently for absorbance determination. The DPPH scavenging activity was expressed as a percentage calculated by [(control absorbance − sample absorbance)/control absorbance] × 100. For determination of antioxidative potency (AOP) with linoleic acid in an iron/ascorbate system, a previously reported procedure was followed (*21*).

Determinations of Crude Protein, Total Free Amino Acid, Sucrose, and Glucose Contents. The freeze-dried whole sprout and sprout component powders were subjected to *n*-hexane extraction to prepare defatted powders following the procedure of Rodriguez et al. (*22*). From the defatted powders, nitrogen content was determined with the Kjeldahl method (*23*) and used to estimate crude protein content ($N \times 5.46$). Total free amino acid contents were determined by the procedures reported by Chiou et al. (*24*). Leucine (Sigma Co.) was used as a reference for quantity estimation.

For sucrose and glucose determination, the procedure of Chiou et al. (*25*) was followed with a minor modification. From each freeze-dried sprout powder, 0.1 g was weighed and deposited into a screw-capped centrifuge tube, replenished with 2.5 mL of deionized water, and followed by homogenization with a polytron equipped with a small aggregate probe for 1 min at 15000 rpm (Polytron PT3000). Then, the homogenate was centrifuged at 19000*g* for 20 min. From the supernatant, 0.5 mL was withdrawn and mixed with 4.5 mL of 60 mM NaOH, membrane filtered (0.2 μm), and subjected to HPLC analysis. An HPLC pump (L-7100 pump, Hitachi Co.) was equipped with an ionic exchange column (RCX 10; Hamilton Co., Reno, NV) and in connection with a RI detector (ERC-7515A; Erma CR. In., Kawaguchi, Japan) to monitor refractive intensity. An isocratic mobile phase using 60 mM NaOH after degassing by passing through a degasser (DP 2010; Uniflows Co., Tokyo, Japan) was employed. The flow rate and injection volume were 1.2 mL/min and 20 μL, respectively. A reference solution

Table 1. Resveratrol Contents of the Cotyledons, Roots, Stems, and Whole Sprouts of Peanut Kernels after Subjection to Germination for Sprout Preparation

cultivar and time (day)	resveratrol content (μg/g freeze-dried solid)[a,b]			
	cotyledon	root	stems	whole sprout
		TNS 9		
0	3.7 ± 0.6[c]			3.7 ± 0.6[c]
3	8.8 ± 2.3[b]	7.3 ± 0.3[b]		6.0 ± 0.9[b]
6	26.1 ± 5.7[a]	7.6 ± 1.5[b]	ND	17.7 ± 3.2[a]
9	32.5 ± 9.1[a]	12.1 ± 1.5[a]	ND	19.9 ± 2.5[a]
		TN 11		
0	4.5 ± 0.5[d]			4.5 ± 0.5[b]
3	15.1 ± 2.4[c]	10.9 ± 2.1[b]		13.4 ± 2.1[ab]
6	29.5 ± 1.9[b]	11.5 ± 0.9[ab]	ND	19.1 ± 3.1[ab]
9	47.1 ± 8.9[a]	18.6 ± 12.6[a]	ND	25.7 ± 10.5[a]
		TN 14		
0	2.3 ± 0.4[d]			2.3 ± 0.4[d]
3	3.6 ± 0.5[c]	4.0 ± 1.7[b]		3.5 ± 0.1[c]
6	6.4 ± 1.8[b]	7.0 ± 0.9[a]	ND	6.4 ± 2.4[b]
9	12.0 ± 3.0[a]	7.9 ± 2.9[a]	ND	11.7 ± 0.2[a]

[a] Each value represents means ± SD ($n = 6$); blank cells, no growth; ND, not detected. [b] Data bearing different superscript letters in the same column for each cultivar were significantly different ($p < 0.05$).

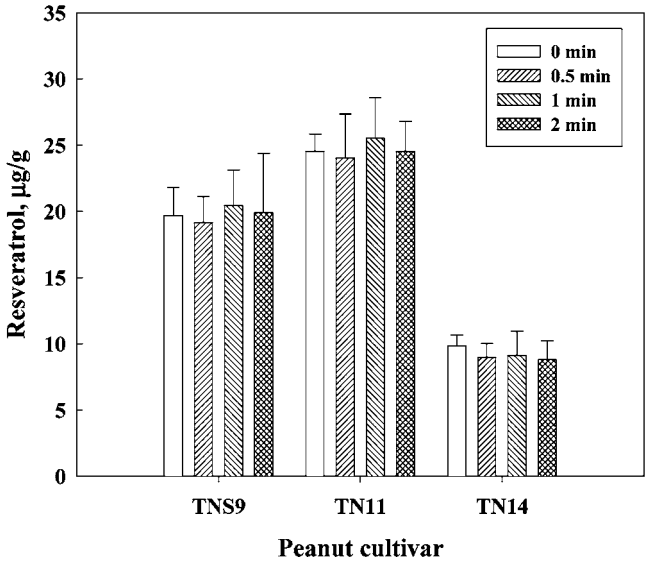

Figure 1. Changes of resveratrol content of peanut sprouts after subjection to boiling water heating for 2 min ($n = 3$).

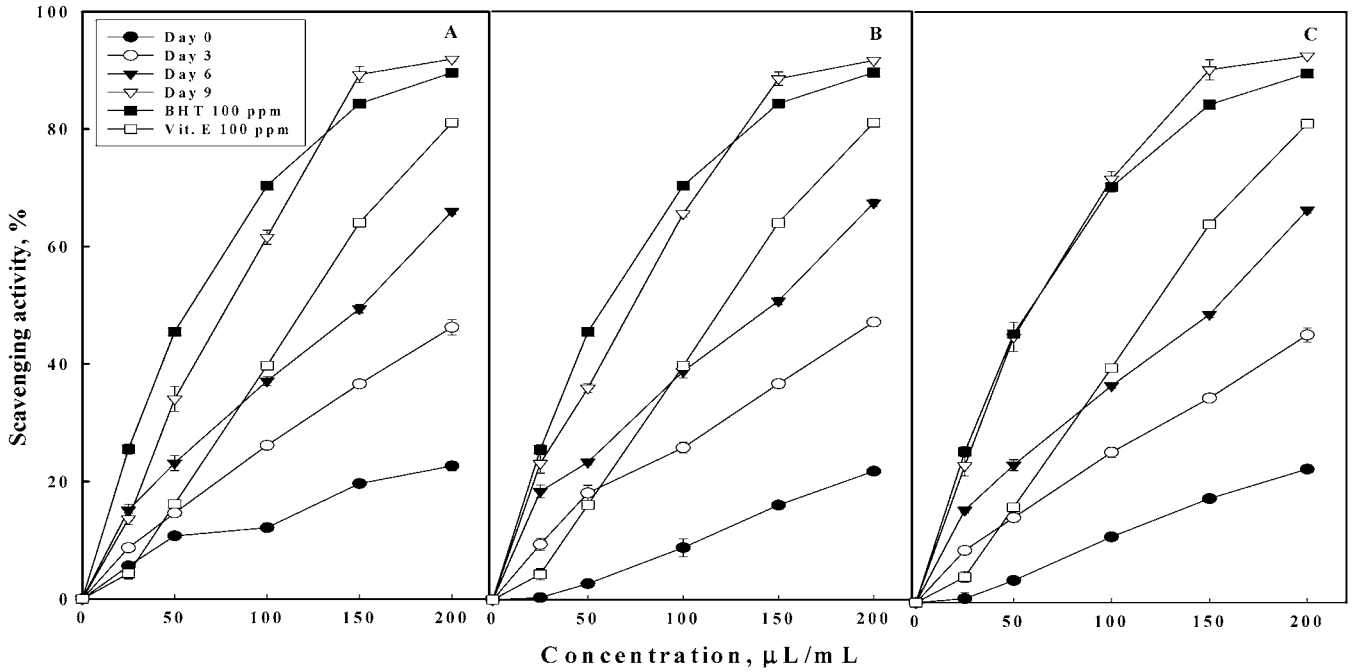

Figure 2. DPPH scavenging activity of the methanol extracts of peanut kernels after subjection to germination for sprout preparation. (**A**) TNS 9, (**B**) TN 11, and (**C**) TN 14 ($n = 3$).

of 60 mM NaOH containing 250 μg/mL sucrose and 100 μg/mL glucose was chromatographed under identical conditions to determine sucrose and glucose contents, respectively, in each sample.

Protein Extration and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS−PAGE). According to the crude protein contents of the defatted peanut kernels (before germination) and sprout powders (after 9 days of germination) determined with the Kjeldahl method as that described above, powders containing 20 mg of crude protein were weighed, deposited into 10 mL centrifuge tubes, and replenished with 4 mL of phosphate buffer (pH 7.9, 0.2 M, sodium salt), after homogenization with a homogenizer (Polytron PT3000) equipped with a small aggregate probe for 1 min at 15 000 rpm. After the tubes were held at the ambient temperature (24−26 °C) for 30 min, the tubes were centrifuged at 19000*g* at 15 °C for 20 min. The supernatant was used as an extracted protein solution for SDS−PAGE following the procedures of Laemmli (*26*) and Chiou et al. (*24*).

Statistics. At least triplicate experiments were conducted. Means of determinations with standard deviations are reported. A Statistica Software (Statistica 1999, StatSoft Co., Tulsa, OK) was used. One-way analysis of variance and Duncan's multiple range method were applied for variation analyses ($p < 0.05$).

RESULTS AND DISCUSSION

After 9 days of germination of the peanut kernels for sprout preparation, germination ratios were 89.7, 87.2, and 93.6% and sprout yields were 256.4, 220.5, and 261.5% (w/w) corresponding to peanut cultivars TNS 9, TN 11, and TN 14, respectively. The lowest sprout yield was TN 11, being closely related to its lowest germination ratio. Obviously, seed viability is essential for the kernels as destined for peanut sprout preparation. When weight distributions of the sprout components including cotyledons, roots, and stems were determined, the freeze-dried weight of cotyledons decreased and weights of roots and stems increased with an increase of germination time. After 9 days of germination, the dry weight distributions of the cotyledons, roots, and stems were 45.0, 25.0, and 30.0% for TNS 9; 53.0, 18.4, and 28.6% for TN 11; and 66.7, 10.7, and 22.7% for TN 14, respectively.

Resveratrol contents of the freeze-dried sprouts and sprout components (cotyledon, root, and stem) after 0, 3, 6, and 9 days of germination are shown in **Table 1**. The resveratrol content of whole sprouts increased significantly ($p < 0.05$) with an increase of germination time. Resveratrol contents of the peanut kernels before germination ranged from 2.3 to 4.5 μg/g as affected by peanut cultivar. After 9 days of germination, it is of merit to notice that resveratrol contents increased up to the range of 11.7−25.7 μg/g. Nevertheless, resveratrol contents deviated considerably as affected by cultivar and individual sprout. This was in agreement with the previous observations that resveratrol content of peanut roots collected from field after peanut harvest deviated considerably among sampling lots (*11*, *13*). In this study, peanut kernels were subjected to germination under an open condition. Different batches or individual kernels must have been challenged by different environmental impacts and resulted in uneven induction of resveratrol biosyntheses.

Among the sprout components after 9 days of germination, the highest resveratrol content was detected in the cotyledons (ranged from 12.0 to 47.1 μg/g), slightly lower in the sprout roots (ranged from 7.9 to 18.3 μg/g), and not detected in the stems. When the sprouts were subjected to heating with boiling water for 2 min, resveratrol contents were mostly unchanged (**Figure 1**). This indicates that sprout resveratrol was fairly stable against heating or cooking such as during preparing a dietary dish.

When the freeze-dried sprouts were subjected to methanol extraction and the extracts were subjected to determination of DPPH scavenging activity, a dose−response to increase the activity by increasing the extract concentration and a longer germination time of the higher scavenging activities of the extracts were observed (**Figure 2**). After 9 days of germination, DPPH scavenging activities of the sprout extracts were close to that of 100 ppm BHT or 100 ppm vitamin E. When the methanol extracts were subjected to AOP determination in prevention of linoleic acid oxidation in an iron/ascorbate system, AOP increased significantly with the time increase of germination (**Figure 3**). The highest AOP was detected by TN 11 and followed in order by that of TNS 9 and TN 14. Apparently, increases of the DPPH scavenging and AOP activities of the

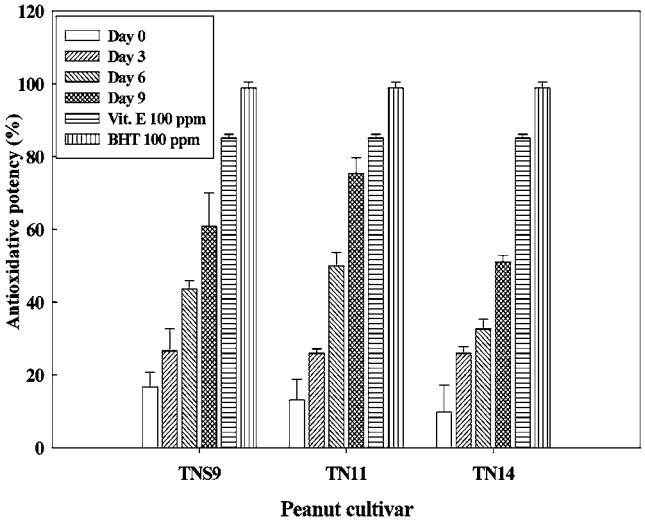

Figure 3. AOP of the methanol extracts of peanut kernels after subjection to germination for sprout preparation ($n = 3$).

Table 2. Changes of Sucrose, Glucose, and Total Amino Acid Contents of Peanut Kernels before and after 9 Days of Germination

	peanut cultivar		
time of germination	TNS 9	TN 11	TN 14
sucrose content (mg/g freeze-dried solid)[a,b]			
before germination	55.9 ± 6.5[b]	52.2 ± 2.5[b]	50.3 ± 5.3[b]
after germination	99.3 ± 6.9[a]	88.0 ± 3.3[a]	116.9 ± 11.0[a]
glucose content (mg/g freeze-dried solid)[a,b]			
before germination[c]	ND	ND	ND
after germination	11.8 ± 1.0	10.5 ± 0.8	11.4 ± 2.0
crude protein content (%, N × 5.46/defatted powder)[a,b]			
before germination	37.6 ± 1.7[a]	37.3 ± 1.1[a]	38.6 ± 0.7[a]
after germination	27.1 ± 0.4[b]	25.6 ± 0.3[b]	27.9 ± 1.0[b]
total soluble amino acid content (mg/g protein)[a,b]			
before germination	0.05 ± 0.01[b]	0.03 ± 0.01[b]	0.03 ± 0.01[b]
after germination	3.22 ± 0.18[a]	2.26 ± 0.06[a]	2.58 ± 0.23[a]

[a] Each value represents means ± SD ($n = 3$). [b] Data bearing different superscript letters in the same column for each cultivar and determination were significantly different ($p < 0.05$). [c] ND, not detected.

methanol extracts were closely relevant to increases of resveratrol contents of the peanut kernels after they were subjected to germination (**Table 1**).

Changes of sucrose and glucose contents of the kernels before and after 9 days of germination are shown in **Table 2**. Before and after 9 days of germination, sucrose contents increased from 55.9 to 99.3, 52.2 to 88.0, and 50.3 to 116.9 mg/g freeze-dried solid for peanut cultivars of TNS 9, TN 11, and TN 14, correspondingly. In a previous study (*27*), the sucrose contents of peanut kernels during subjection to germination decreased in the early 72 h of germination time and increased afterward. Glucose was not detected in the kernels before germination. However, after 9 days of germination, glucose contents increased remarkably to the range from 10.5 to 11.8 mg/g freeze-dried solid as affected by peanut cultivar.

As affected by germination, crude protein contents of the peanut kernels decreased and total free amino acid contents increased (**Table 2**). This was in agreement with the observations of Basha et al. (*14*) and Chiou et al. (*27*) who reported that total free amino acid contents of peanut kernels increased during germination and the increase of total free amino acid content is closely relevant to the decrease of protein content.

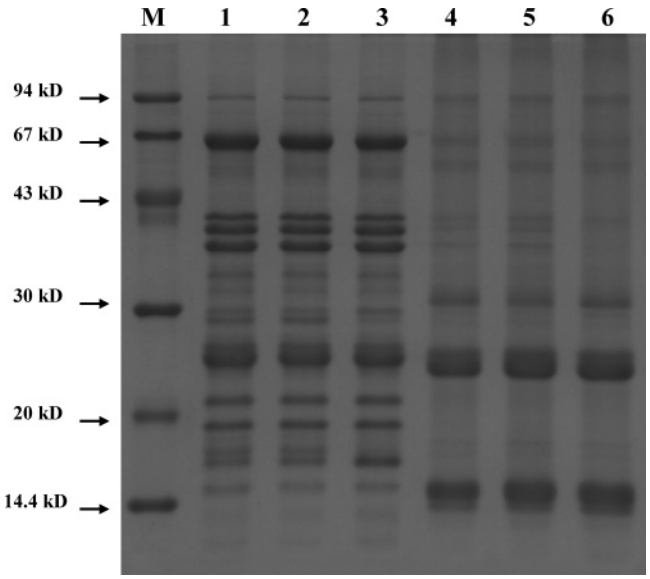

Figure 4. SDS–PAGE protein patterns of peanut kernels before and after 9 days of germination. M, protein marker; 1, TNS 9 before germination; 2, TN 11 before germination; 3, TN 14 before germination; 4, TNS 9 after germination; 5, TN 11 after germination; and 6, TN 14 after germination.

Changes of peanut proteins were further supported by the changes of SDS−PAGE protein patterns shown in **Figure 4**. Because the same amounts of protein were loaded for electrophoresis, qualitative comparison among the patterns was available. There was a band, with a molecular mass ca. 30 kDa, that appeared in the extracted proteins of sprouts after 9 days of germination. In general, the patterns indicated that most large protein molecules in the kernels were degraded after germination. This was in agreement with the observations that SDS−PAGE profiles of some legumes including peanut vary significantly during germination (*27*, *28*).

In conclusion, peanut sprouts germinated from peanut pods or kernels left in the fields after peanut harvest are conventionally collected by farmers as a vegetable. Logically, germination of peanut kernels to produce sprouts as a fresh produce is of perspective. In this study, resveratrol contents and antioxidative activities that increased significantly with an increase of germination time may provide some health benefits to the sprout consumers. In addition, significant increases of sucrose, glucose, and total free amino acid contents of the peanut kernels after 9 days of germination may enhance sprout taste and flavor preference. In accordance with the bioactive and chemopreventive significance of resveratrol, these features render peanut sprouts with merits to be developed as a functional vegetable. From a practical viewpoint, peanut sprouts could be produced from a home−cottage scale to a commercial scale.

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