Antioxidant activity, total phenolic, and resveratrol content in five cultivars of peanut sprouts

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ABSTRACT

Objective: To investigate the change in total phenolic compounds, antioxidant activity, and resveratrol content of five different germinated peanut cultivars.

Methods: The germinated sprouts of five peanut cultivars (Kalasin1, Kalasin2, Konkaen, Konkaen4, and Tainan9) were extracted with 80% ethanol and collected as crude extract. The antioxidant capacities were determined with 2,2-diphenyl-1-picrylhydrazyl and ferric ion reducing antioxidant power method. The total phenolic compound was measured using the Folin–Ciocalteau assay. The qualification and quantification of resveratrol was performed by high performance liquid chromatography method.

Results: Among the five cultivars, a three-day germination of Kalasin1 showed the highest phenolic content [(40.67 ± 2.62) mg gallic acid/g dry weight], expressed the highest 2,2-diphenyl-1-picrylhydrazyl antioxidant value [(80.51 ± 1.47) mmol/L Trolox/g dry weight], and ferric ion reducing antioxidant power antioxidant value [(171.33 ± 8.59) mmol/L ascorbic acid/g dry weight]. However, the high performance liquid chromatography result of Kalasin2 significantly increased to the highest resveratrol content of (6.44 ± 1.26) µg/g dry weight on the second day of germination.

Conclusions: The variation of phytochemical content in the peanut sprout is due to the effect of the peanut cultivar and the germination period.

1. Introduction

Peanut (Arachis hypogaea) is an annual herbaceous plant which belongs to the Fabaceae family. Peanut is a legume of nutrient abundance and contains a wide variety of chemical constituents such as proteins, carbohydrates, fibers, fats, niacin, folate, thiamine, arachidic acid, flavonoids, magnesium, and phosphorus. There are vast varieties of peanut growing in Thailand with different backgrounds and characteristics. It has been reported that peanuts possess diverse pharmacological activities such as antiviral, antimicrobial, anti-inflammatory, anticancer, antihypertension, antiproliferative, cardiovascular disease protection, and neuroprotection [1–3].

The seed germination process produces a new generation of plant. Complex biochemical changes occur during hydration and following sprouting. As reported by Kim et al., the sprouts are rich in phytochemicals, proteins, vitamins, and minerals [4]. These nutrients are essential for human health and have been used in health promoting food. Several studies have reported that wide varieties of phenolic substances such as resveratrol, arachidin-1, and piceatannol have been found in peanut and peanut sprout [5–7]. Phenolic compounds are generally secondary metabolite produced in plants and they might be correlated to antioxidant activity which has been shown to retain various biological benefits [8]. These phenolic compounds have a protective effect against oxidative reactions such as reducing...
activity, metal chelating properties, and a hydrogen donor function. Thus, these compounds appear to be antioxidants [9].

Resveratrol (3,4′,5-trihydroxystilbene) is a major natural polyphenolic compound found in peanuts and peanut sprouts. Resveratrol belongs to the stilbene group and is synthesized by the enzyme resveratrol synthase. The plant produces resveratrol as a defense mechanism against pathogen infection, UV radiation, and other mechanical stress damage [10,11]. Recently, there has been a great deal of focus on resveratrol due to its health benefits including anti-aging [12], anticancer [13], anti-inflammatory [14] and the prevention of cardiovascular disease [15]. The plausible mechanism of cardioprotection is the platelet aggregation inhibition of resveratrol [16]. Krohne et al. have demonstrated that many retinal diseases have higher levels of lipid peroxidation and decreased antioxidant concentration in the cells [17]. This retinal degenerative symptom could be reduced by the inhibition of resveratrol through oxidative induced apoptosis in human retinal pigment epithelium. Thus, the use of resveratrol as a health promoting dietary supplement is rapidly increasing in today’s market.

The determination of resveratrol in different peanut cultivars showed that the resveratrol content varied between different peanut cultivars [18]. In addition, the peanut sprout had higher resveratrol content than the ungerminated peanut kernel, depending on the peanut cultivar. Compared to the different plant parts, higher resveratrol content was found in the cotyledons, but lower in the roots, and not detected in the stems [5].

The objective of this study is to investigate the antioxidant, total phenolic compounds, and resveratrol content of various germinated peanut cultivar seeds (Kalasin1, Kalasin2, Konkaen, Konkaen4, and Tainan9) growing in Thailand. These five cultivar seeds were subjected to germination for 4 days. The ungerminated peanut kernels were discarded. The germinated peanut sprouts were collected and subsequently dried in an oven at 60 °C. The dried peanut sprouts were pulverized to prepare the whole sprout powders, followed by 80% ethanol extraction for 24 h. The solvent evaporation was carried out using a rotary evaporator at 40 °C and collected as crude extract.

2.2. Peanut sprout extraction

The five cultivars of peanuts; Kalasin1, Kalasin2, Konkaen, Konkaen4, and Tainan9 used in this study were kindly provided by Associate Professor Suwaree Saijeen (Rajamangala Institute of Technology Lanna, Phitsanulok, Thailand).

Seeds of various peanut cultivars were soaked in the 0.5% NaCl solution for half an hour and subsequently submerged in tap water for 3 h to rehydrate. Seeds were then germinated in the dark under 15 min of spray tap water with pauses of 15 min for 1–4 days. The ungerminated peanut kernels were discarded. Only the germinated peanut sprouts were collected and subsequently dried in an oven at 60 °C. The dried peanut sprouts were extracted using 20 mmol/L FeCl3 in 300 mmol/L acetate buffer pH 3.6. The antioxidant capacity based on the decolorization of DPPH was described by the Kenny protocol [19]. Briefly, 10 μL of crude extract (10 mg/mL) was added to 100 μL of 0.12 mmol/L DPPH radical, along with ethanol, to provide a total reaction volume of 200 μL. Ethanol was used as a blank and the reaction mixture was immediately mixed and incubated in the dark for 30 min. The pale yellow color of the reduced DPPH radical was measured at optical density of 515 nm at room temperature. Measurements were recorded in triplicate and Trolox was used as the standard. The result of antioxidant was expressed as Trolox equivalent antioxidant capacity (TEAC) (mmol/L Trolox/g dry weight).

2.3. DPPH radical scavenging assay

The reaction was incubated at room temperature for 5 min. The absorbance was read at 593 nm in three replicate experiments. The total reducing capacity of the FRAP assay was calculated using ascorbic acid as the standard. The result was expressed as ascorbic acid equivalent (AAE) (mmol/L ascorbic acid/g dry weight).

2.4. Ferric ion reducing antioxidant power (FRAP) assay

Based on the reduction of ferric to ferrous ion and formation of ferrous-tripyridyltriazine (Fe(II)-TPTZ) complex at low pH, the antioxidant powder was measured at optical density of 593 nm. The intense blue color performed in accordance with the presence of the antioxidant, which acted as a reductant in the assay reaction. The FRAP assay was conducted by modifying the protocol from Müller et al. [20]. Briefly, 2 μL of crude extract was mixed with 198 μL of fresh FRAP working solution (10 mmol/L TPTZ and 20 mmol/L FeCl3 in 300 mmol/L acetate buffer pH 3.6). The reaction mixture was immediately mixed and incubated at room temperature for 5 min. The absorbance was read at 593 nm in three replicate experiments. The total reducing capacity of the FRAP assay was calculated using ascorbic acid as the standard. The result was expressed as ascorbic acid equivalent (AAE) (mmol/L ascorbic acid/g dry weight).

2.5. Total phenolic compound assay

The total phenolic compound was determined using the Folin–Ciocalteau method. Briefly, the reaction was carried out using 2 μL crude extract (10 mg/mL) mix to 50 μL Folin reagent and then adding 50 μL sodium carbonate (20% w/v) solution. The reaction was incubated at room temperature for 30 min in the dark and the absorbance was measured at 765 nm. Gallic


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acid was used as the standard and the result was expressed as gallic acid equivalent (GAE) (µg gallic acid/g dry weight). Each sample was done in three replicates.

2.6. HPLC

The qualification and quantification of resveratrol was performed using HPLC method described by Lee et al., with some modifications [21]. The peanut sprout crude extract was filtered through a 0.45-µm filter before injection into HPLC. The separation was performed on a C18 reverse phase column (Luna 5 µm C18 (2) 100 A Column, Phenomenex). The mobile phase consisted of acetonitrile:water (35:65, v/v) and was run at a constant flow rate of 1 mL/min. Chromatograms were detected using a UV detector at 306 nm. The trans-resveratrol was used as the standard to plot the curve between the standard concentration and average peak area.

2.7. Statistical analysis

Analysis of the experimental data was performed in triplicate and reported as mean ± SD. Statistical One-way ANOVA was calculated using SPSS software version 17.0. Differences were considered statistically significant at the P < 0.05 level.

3. Results

3.1. Total phenolic compounds and antioxidant activity

The five released peanut cultivars (Kalasin1, Kalasin2, Konkaen, Konkaen4, and Tainan9) were germinated for 1–4 days and evaluated for their phenolic compound contents using the Folin–Ciocalteau method. Evaluation of the antioxidant capacity was done by DPPH and FRAP assay. In this study we focused on an extended germination period of 1–4 days with the aim of determining a powerful period of phenolic and antioxidant compound synthesis in different peanut cultivar sprouts as shown in Table 1.

3.1.1. Kalasin1 peanut cultivar

The phenolic compound of the Kalasin1 cultivar significantly increased from one to three days germination and subsequently decreased on the fourth day. Among the five cultivars, the three-day germination period of Kalasin1 showed the highest phenolic compound content [(40.67 ± 2.62) µg gallic acid/g dry weight]. According to the total phenolic compound content, the three day germination of the Kalasin1 cultivar showed the highest antioxidant values in DPPH [(80.51 ± 1.47) mmol/L Trolox/g dry weight] and FRAP [(171.33 ± 8.59) mmol/L ascorbic acid/g dry weight].

3.1.2. Kalasin2 peanut cultivar

The phenolic content and antioxidant activity pattern of the Kalasin2 cultivar was distinct from that of Kalasin1. In contrast to Kalasin1, a significant decrease in the total phenolic compound on the third day of germination [(19.45 ± 2.16) µg gallic acid/g dry weight] was observed for the Kalasin2 cultivar. Later, the phenolic content increased to (28.08 ± 4.22) µg gallic acid/g dry weight on the fourth day of germination. On the other hand, the DPPH antioxidant was constant during the first three days of germination and increased on the fourth day to (61.62 ± 10.85) mmol/L Trolox/g dry weight. The FRAP antioxidant capacity exhibited a high value of (96.22 ± 3.82) mmol/L ascorbic acid/g dry weight, and (98.65 ± 7.07) mmol/L ascorbic acid/g dry weight on the second and fourth day of germination, respectively.

3.1.3. Konkaen peanut cultivar

The Konkaen cultivar showed a gradually decreasing phenolic compound content after an extended germination.

Table 1

<table>
<thead>
<tr>
<th>Peanut type</th>
<th>Peanut cultivar</th>
<th>Germination time (days)</th>
<th>Phenolic content (µg gallic acid/g dry weight)</th>
<th>Antioxidant capacity (mmol/L ascorbic acid/g dry weight)</th>
<th>Resveratrol (µg/g dry weight)</th>
</tr>
</thead>
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<tr>
<td>Valencia</td>
<td>Kalasin1</td>
<td>1 day</td>
<td>22.96 ± 2.53</td>
<td>73.74 ± 1.35</td>
<td>1.40 ± 0.04</td>
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<tr>
<td></td>
<td></td>
<td>2 day</td>
<td>30.81 ± 1.76</td>
<td>57.93 ± 9.54</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 day</td>
<td>40.67 ± 2.62</td>
<td>80.51 ± 1.47</td>
<td>0.52 ± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 day</td>
<td>23.75 ± 2.33</td>
<td>57.13 ± 2.13</td>
<td>0.60 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Kalasin2</td>
<td>1 day</td>
<td>35.99 ± 2.54</td>
<td>46.85 ± 5.20</td>
<td>1.57 ± 0.82</td>
</tr>
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<td></td>
<td></td>
<td>2 day</td>
<td>33.15 ± 1.50</td>
<td>47.03 ± 3.46</td>
<td>6.44 ± 1.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 day</td>
<td>20.49 ± 1.61</td>
<td>45.01 ± 4.95</td>
<td>2.78 ± 1.51</td>
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<tr>
<td></td>
<td></td>
<td>4 day</td>
<td>28.08 ± 4.22</td>
<td>61.62 ± 10.85</td>
<td>0.31 ± 0.02</td>
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<td>Konkaen</td>
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<td>33.76 ± 1.99</td>
<td>53.44 ± 5.73</td>
<td>1.20 ± 0.57</td>
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<td></td>
<td></td>
<td>2 day</td>
<td>22.49 ± 1.53</td>
<td>53.70 ± 6.64</td>
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<tr>
<td></td>
<td></td>
<td>3 day</td>
<td>27.34 ± 2.04</td>
<td>47.41 ± 7.14</td>
<td>0.78 ± 0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 day</td>
<td>12.06 ± 1.21</td>
<td>44.48 ± 4.75</td>
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</tr>
<tr>
<td></td>
<td>Konkaen4</td>
<td>1 day</td>
<td>36.85 ± 3.46</td>
<td>64.96 ± 8.71</td>
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<td></td>
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<td>2 day</td>
<td>24.36 ± 2.70</td>
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<td>0.57 ± 0.32</td>
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<td></td>
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<td>3 day</td>
<td>28.39 ± 2.49</td>
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<td>17.87 ± 3.55</td>
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<tr>
<td></td>
<td>Spanish Tainan9</td>
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<td>15.65 ± 1.97</td>
<td>29.36 ± 1.47</td>
<td>3.69 ± 1.06</td>
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<tr>
<td></td>
<td></td>
<td>2 day</td>
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<td>29.89 ± 2.60</td>
<td>1.78 ± 0.87</td>
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<td>41.93 ± 7.33</td>
<td>0.36 ± 0.09</td>
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<td></td>
<td></td>
<td>4 day</td>
<td>28.72 ± 1.51</td>
<td>47.91 ± 0.76</td>
<td>0.63 ± 0.20</td>
</tr>
</tbody>
</table>

The data represent the mean ± SD of triplicate assay for each sample. The mean ± SD within each peanut cultivar in the same column followed by the same letter are not significantly different at P < 0.05.
time. Even though the phenolic content on the third day of germination is not the highest value, the antioxidant showed the highest value on the third day of germination [DPPH antioxidant (64.96 ± 8.71) mmol/L Trolox/g dry weight and FRAP antioxidant (84.20 ± 1.80) mmol/L ascorbic acid/g dry weight]. On the fourth day of germination, the Konkaen cultivar showed the lowest phenolic compound content [(12.06 ± 1.21) mg gallic acid/g dry weight] of the five cultivars. The antioxidant assays by the DPPH method also decreased to (44.48 ± 4.75) mmol/L Trolox/g dry weight, and FRAP of (52.43 ± 0.41) mmol/L ascorbic acid/g dry weight on the fourth day of germination.

3.1.4. Konkaen4 peanut cultivar

The total phenolic content, as well as the DPPH and FRAP antioxidant activity of the Konkaen4 cultivar concomitantly decreased after the germination period. The highest values were detected on the first day of germination [phenolic content (36.85 ± 3.46) mg gallic acid/g dry weight, DPPH antioxidant (64.43 ± 3.94) mmol/L Trolox/g dry weight, and FRAP antioxidant (101.07 ± 2.97) mmol/L ascorbic acid/g dry weight]. On the final germination day, the phenolic content and antioxidant values significantly dropped to (17.87 ± 3.55) mg gallic acid/g dry weight for phenolic content, (43.60 ± 0.95) mmol/L Trolox/g dry weight for DPPH, and (67.87 ± 5.14) mmol/L ascorbic acid/g dry weight for FRAP.

3.1.5. Tainan9 peanut cultivar

In this study, Tainan9 is the only cultivar taken from a Spanish cultivar group. The pattern of phenolic content and antioxidant activity was different from the other four cultivars. The phenolic content and antioxidant activity steadily increased on the first and second day of germination. During the extended germination time all values significantly increased and reached the highest level [phenolic content (28.72 ± 1.51) mg gallic acid/g dry weight, DPPH antioxidant (47.91 ± 0.76) mmol/L Trolox/g dry weight, and FRAP antioxidant (113.09 ± 5.44) mmol/L ascorbic acid/g dry weight] on the fourth day of germination. Interestingly, the antioxidant measured by FRAP dramatically increased to (113.09 ± 5.44) mmol/L ascorbic acid/g dry weight, expressed as approximately four times compared to (30.63 ± 2.41) mmol/L ascorbic acid/g dry weight on the first day of germination. This indicated that antioxidant compounds expressing high reducing ability might be present at this germination stage.

3.2. Resveratrol content

Resveratrol is a natural stilbene phytoalexin with benefits to human health. The results in Table 1 demonstrated the resveratrol content of five peanut cultivars measured by the HPLC method. The HPLC chromatograms with the highest level of resveratrol content for each cultivar of peanut sprout crude extract was presented in Figure 1. Three peanut cultivars:
Kalasin1, Konkaen4, and Tainan9 indicated a similar pattern, producing the highest resveratrol content on the first day of germination and a decrease in germination time. The highest resveratrol content on the first day of germination was $(1.40 \pm 0.04) \mu$g/g dry weight, $(2.88 \pm 1.23) \mu$g/g dry weight, and $(3.69 \pm 1.06) \mu$g/g dry weight for Kalasin1, Konkaen4, and Tainan9, respectively. Although resveratrol was effective for the in vitro scavenging activity, some data, such as that for the third germination day of the Kalasin1 cultivar, showed a contradictory result.

The Kalasin2 and Konkaen cultivars also showed a similar pattern of resveratrol production. The resveratrol significantly increased to its highest level on the second day of germination and decreased on the next germination day. As shown in Table 1, the Kalasin2 cultivar expressed $(6.44 \pm 1.26) \mu$g/g dry weight and the Konkaen cultivar produced $(2.25 \pm 1.00) \mu$g/g dry weight resveratrol on the second day of germination.

4. Discussion

With the entire phenolic content and antioxidant activity result of five peanut cultivars, each cultivar showed diverse patterns during seed germination. As the data shown from the result, FRAP value of Kalasin1 peanut cultivar at three days germination increased two-fold from the first day. This result suggested that most of the phenolic compounds at three days germination determined in the Kalasin1 cultivar could be related to their antioxidant activity. For the Kalasin2 peanut cultivar, the FRAP antioxidant activity on the second and fourth day of germination was increased to a greater extent than the phenolic content. This can be explained by different structural compounds other than those in the phenolic group contributing to the reduction in antioxidant capacity, since the FRAP assay relies on the redox reaction of electron transfer and indicates the reducing ability of antioxidant compounds [22]. The only Spanish cultivar group in this study; Tainan9 expressed the different pattern of the phenolic content and antioxidant activity from other cultivars on each germination day. The antioxidant activity of Tainan9 cultivar was significantly increased during the extended germination time. This result was in accordance with the study by Wang et al. which reported increased scavenging activity for the Tainan9 cultivar with a longer germination time [5]. The difference in phenolic content and antioxidant value between five peanut cultivar sprouts may be strongly influenced by peanut genotype, which produced different complicated structural compositions in each. A wide-ranging biochemical process occurred during seed germination, leading to plant changes during secondary metabolite production [23]. This could affect the intrinsic phenolic content and antioxidant activity profile of the plant.

In order to analyze the relationship of total phenolic compounds and antioxidant activity through the germination period, the correlation analysis was observed among the total phenolic content and various assay methods of antioxidant capacity. A positive correlation was inspected between the phenolic content and DPPH method ($r = 0.609$). This was in accordance with the study by others [24] which found a strong correlation between the total phenolic compound and antioxidant activity determined by the DPPH method. Previous reports have revealed that the phenolic content increased correspondingly with higher antioxidant activity; therefore phenolic compounds were the dominant antioxidant components. Although the correlation result in this study was not as high as the previous report, this may be explained by the synergistic antioxidant response not only arising from the phenolic compounds, but possibly from the interaction of other phytochemicals in the crude extract [25]. Moreover, the antioxidant capacity of phenolic compounds was determined by the compound structures. A high correlation of total phenolic content and small molecular weight chemical compounds has also been reported [26]. Steric accessibility of the antioxidant molecule to the DPPH radical represents an important mechanism for antioxidant assessment. A report by Prior et al. showed that small molecules can access the DPPH radical better than larger ones and appeared to change color more quickly [27]. Therefore, low molecular weight bioactive compounds assessed by the DPPH method in the present study positively correlated with the total phenolic contents.

Similarly, the phenolic content and FRAP method also showed positive correlation ($r = 0.636$). Fidrianny et al. reported high correlation among total phenolic and FRAP antioxidant assays in Cucurbitaceae leaves extracts [28]. The FRAP assay is based on electron transfer, which measures the reducing power of antioxidant compounds, but is unable to detect the radical quenching antioxidant mechanism. This reducing power is considered to be associated with the degree of hydroxylation in the polyphenolic compound.

Additionally, the positive correlation between DPPH and FRAP methods was also noted ($r = 0.773$). Likewise, a strong correlation was found between DPPH and FRAP assays [29]. It was apparent that the two different antioxidant assays share a basic similar mechanism. Both DPPH and FRAP methods detect the ability of an electron transfer antioxidant to reduce any oxidant compound. It is suggested that there is no single assay for measuring overall antioxidant capacity since individual assays illustrate different aspects of antioxidant behavior. The evaluation of widespread antioxidant capacity may need multiple assays in order to improve the overall antioxidant profile of plant extracts.

It has been speculated that resveratrol is an effective in vitro antioxidant and radical scavenging activity [30]. The third germination day of Kalasin1 expressed the highest phenolic content and antioxidant activity, while the resveratrol content was detected at only $(0.52 \pm 0.17) \mu$g/g dry weight. The high phenolic content and antioxidant activity might indicate that substances other than resveratrol could also act synergistically as a combined action and result in more antioxidant activity than that expected from resveratrol alone [25]. Vieira et al. also reported on the synergistic effects of the combined extracts, either phenolic or polysaccharidic to enhance antioxidant activity [31]. In addition, other substances could also act synergistically with the phenols; and therefore resveratrol could not be the only substance responsible for antioxidant activity. Among the five cultivars, Kalasin2 exhibited the highest resveratrol content of $(6.44 \pm 1.26) \mu$g/g dry weight, which was four times higher than the first germination day. It was found that sprouting peanuts showed an increasing value of resveratrol [32]. A previous report indicated that the resveratrol content varied according to the different peanut cultivars and growth states of an individual sprout. In addition, different parts of peanut sprouts such as cotyledons, roots, and stems also showed different amounts of resveratrol [5]. Along with the results of this study, suitable peanut cultivars and appropriate germination times for sprouting peanuts should be taken into consideration when increasing the resveratrol content.
Peanuts are available sources of potential phytochemical compounds such as stilbenes and flavonoids. The germination of peanuts is a crucial step towards producing a bioactive compound for plant growth. Peanut sprouts provide an excellent source of bioactive compound generation with potential phenolic compounds and antioxidant activity. The germination stage of peanuts is important since the phenolic content and antioxidant activity at each germination stage varies. The variation in phytochemical content may be due to the effect on peanut cultivars at the germination stage, the storage conditions, growing region, and growing season. As an important stilbene phytoalexin, resveratrol found in peanuts is beneficial to human health. In the present study, three peanut cultivars: Kalasin1, Konkaen4, and Tainan9 produced the highest resveratrol content on the first day of germination and showed a decrease in germination time. However, the resveratrol content in the Kalasin2 and Konkaen cultivars increased to the highest level on the second day of germination and decreased on the next germination day. Among the five cultivars, Kalasin2 exhibited the highest resveratrol content of (6.44 ± 1.26) µg/g dry weight. The difference in the phenolic content and antioxidant value between five peanut cultivar sprouts may be strongly influenced by the complicated structural composition of each genotype. Other than resveratrol, certain substances in different cultivars of the peanut sprout crude extract may act synergistically to enhance, or antagonistically to reduce the antioxidant activity. Due to the effectiveness and bioactive properties of resveratrol, peanut sprouts possess interesting features for development into a value-added functional food to enhance health benefits. Further investigation may be needed in order to enhance the production of resveratrol in peanuts.

Conflict of interest statement

We declare that we have no conflict of interest.

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References


