

## Effect of germination on total phenolic, tannin and phytic acid contents in soy bean and peanut

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**Abstract:** Legume is a plant in the family of *Fabaceae* or *Leguminosae* that rich in protein, carbohydrate, dietary fibre, and minerals. Germination of legume causes some important changes in the biochemical and a nutritional characteristic of the legumes that may be beneficial to human's health and nutritional status. This study was carried out to determine the effect of germination on total phenolic, tannin and phytic acid contents of soy beans and peanut. The process of germination was carried out by soaking legumes in water for 6 hours, followed by germinating them in wet muslin cloth for 48 hours. After germination, samples were dried and stored in refrigerator before analysis. Total phenolic, tannin and phytic acid were determined spectrophotometrically. Total phenolic contents were decreased significantly ( $p < 0.05$ ) in germinated peanut and soy bean while significant decreased ( $p < 0.05$ ) of tannin content was found in germinated peanut. Phytic acid content was decreased significantly ( $p < 0.05$ ) in germinated soy bean. Germination reduced total phenolic, tannin and phytic acid contents more in peanut compared to soy bean sample. The decreased in total phenolic, tannin and phytic acid content was due to enzymatic changes during germination period in seeds. Germinated beans can be incorporated with wheat-based food product such as bread or pasta to improve nutrition content.

**Keywords:** Germination, total phenolic, tannin, phytic acid, soy bean, peanut

### Introduction

Legume is a plant in the family of *Fabaceae* (or *Leguminosae*), or a fruit of these specific plants, characterized by edible seeds, borne in pods that often open along two seams, by pea-shaped flowers, and by compound stipulate leaves (Mazur *et al.*, 1998). It is one of the important sources of protein, carbohydrate, dietary fibre and minerals (Tharanathan and Mahadevamma, 2003). Soy beans and peanut are well known of their high oil content. Their fat characteristics and fatty acid components have been extensively investigated. On average, soy bean contains 18.0-22.0% oil while peanut contains 40-50% oil (Yoshida *et al.*, 2003; Yoshida *et al.*, 2005). However, other leguminous seeds were reported to be low in lipid where the percentage of lipids in other leguminous seeds is between 1 and 2% (Akpınar *et al.*, 2001).

Germination is a natural process occurred during growth period of seeds in which they meet the minimum condition for growth and development (Sangronis *et al.*, 2006). During this period, reserve materials are degraded, commonly used for respiration and synthesis of new cells prior to developing embryo (Vidal-Valverde *et al.*, 2002). The process starts with the uptake of water (imbibitions) by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually the radical (Bewley and

Black, 1994).

Several studies on the effect of germination on legumes found that germination can increase protein content and dietary fiber, reduce tannin and phytic acid content and increase mineral bioavailability (Rao and Prabhavathi, 1982; Ghanem and Hussein, 1999; Ghavidel and Prakash, 2006). Germination also was reported to be associated with increase of vitamin concentrations and bioavailability of trace elements and minerals (El-Adawy *et al.*, 2002). Kaushik *et al.* (2010) found that germination improved calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid contents.

Tannin in plant is involved in defence mechanism to environmental attack (Okuda *et al.*, 1992). It was reported to have potential antioxidant properties in faba beans mainly due to chain-breaking ability rather than to chelating activity with transition metals (Carbonaro *et al.*, 1996). Phytic acid or phytate when in salt form is the principal storage form of phosphorus in plant tissues (Kumar *et al.*, 2010). It was formed during maturation of the plant seed and represents 60-90% of total phosphate in dormant seeds (Loewus, 2002). In spite of many negative aspects on human health, there were reports that shown phytate has some favorable effects such as anticarcinogen (Shamsuddin, 2002; Vucenik and Shamsuddin, 2003) and lower blood glucose by reducing the rate of starch digestion and slowing gastric emptying (Thompson,

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1993).

This study was aimed to determine the effect of germination on total phenolic, tannin and phytic acid contents of soy bean and peanut. Soy bean and peanut were selected as both are economically important legume worldwide (Zhu *et al.*, 2005; Yoshida *et al.*, 2005; Kim *et al.*, 2006). Asia region has the highest consumers of soy bean with 2.21 kg/capita/year while Africa has the highest consumers of peanut with 1.23 kg/capita/year, in 2007 (FAO, 2011). Specifically, soy bean consumption was highest in Japan and South Korea at 8.19 and 7.49 kg/capita/year, respectively while Chad and Burkina Faso have the highest consumers of peanut at 11.81 and 10.06 kg/capita/year, respectively. Availability of such data on germinated soy and peanuts may be helpful in providing nutritional information of the alternative forms of these legumes.

## Materials and Methods

### Samples

Dried soy bean and peanut were purchased from a hypermarket in Seri Kembangan, Selangor, Malaysia. Legumes were stored in refrigerator at 4°C before germination.

### Germination of soy bean and peanut

Samples were washed and cleaned with tap water before soaked for 6 hours at room temperature (28°C). After 6 hours, samples were put under wet muslin cloth and left germinated for 48 hours at room temperature (28°C) without direct contact with sun light (Yasminet *et al.*, 2008).

### Extraction of germinated soy bean and peanut

Extraction was done following the method of Xu and Chang (2008) with a slight modification. About 0.5 g ground germinated samples were accurately weighed in centrifuge tubes. Five millilitres of acetone/water (50:50 v/v) extraction solvent were added to the samples. The tubes were capped and vortex (Boeco, Germany) for 15 min before centrifuged at 3000 rpm for 10 min using centrifuge model Rotofix 32 A (Hettich, Germany). After that, samples were stored at 4°C in the dark before analysis.

### Total phenolics

Total phenol content was determined using the method of Makkar *et al.* (1993). Samples (50 µL) were put in test tubes and the volume was made up to 500 µL using distilled water. Then, 250 µL of Folin-Ciocalteu reagent was added into the test tube followed by 1.25 mL of sodium carbonate solution.

The tubes were vortexed before incubated in the dark for 40 min. Absorbance was read at 725 nm using spectrophotometer (Shimadzu, Australia).

### Tannin content

Using the method of Makkar *et al.* (1993) for determination of non-tannin phenolics, 100 mg PVPP was weighed in test tubes before being added with 1.0 mL distilled water and 1.0 mL of the extracted sample. The tubes were vortexed before kept at 4°C for 15 min. Then, the tubes were vortexed again before centrifuged at 3000 rpm for 10 min. The supernatant was collected and measured for absorbance at 725 nm using spectrophotometer (Shimadzu, Australia). The tannin content was calculated as follows:

$$\text{Total phenolics} - \text{non-tannin phenolics} = \text{tannin}$$

Total phenolic and tannin content were expressed as gallic acid equivalents through the calibration curve of gallic acid (Sigma, USA) with the concentration range of 0-100 mg/mL.

### Phytic acid

Dried samples were transferred into 100 ml conical flasks and added with 40 ml of sodium sulphate in 1.2% HCl. Flasks were capped and shaken vigorously for 2 hour on a rotator at ambient lab temperature (28°C). The supernatant was filtered through qualitative filter paper. Then, 10 ml of filtered extract were diluted to 30 ml with distilled water after mixing with 1 ml of 30 g/l NaOH and was passed through an anion resin column (resin, AG1-X4, ~100-200 mesh, Bio-Rad Laboratory, Inc., CA). Eluate from the resin was eluted with 0.7 mol/l NaCl to 25 ml. Four ml Postcolumn reagent (0.03% FeCl<sub>3</sub> solution + 0.3% sulfosalicylic acid) was added into 5 ml of the collected eluate and centrifuged at 3000 rpm for 10 min. The supernatant was measured for absorbance at 500 nm using spectrophotometer (Shimadzu, Australia). Standard curve was prepared using phytate standard (Sigma, USA) with the range of 0-100 mg/100 g (Ma *et al.*, 2005).

### Statistical analysis

The statistics software Statistical Package for Social Sciences (SPSS) version 16.0 for Windows was used to analyze total phenolics, tannin and phytic acid content and results were expressed as mean ± standard deviation (SD). Comparison of the difference in total phenolics, tannin and phytic acid content between germinated and non-germinated soy bean and peanut was analyzed using one-way analysis of variance (ANOVA) analysis. Significant

difference was determined at  $p < 0.05$ .

## Results and Discussion

### Total phenolics and tannin content

Table 1 presents the total and non-tannin phenolics and tannin in germinated and non-germinated soy bean and peanut. Among the four samples, non-germinated peanut contained the most total phenolics content with  $43.79 \pm 2.381$  followed by germinated peanut ( $18.777 \pm 1.698$ ), non-germinated soy bean ( $13.767 \pm 0.294$ ) and germinated soy bean ( $10.673 \pm 0.434$ ). Comparing the germinated and non-germinated soy bean, the total phenolics content was decreased significantly ( $p < 0.05$ ) from  $13.767 \pm 0.294$  to  $10.673 \pm 0.434$  with 22.47% reduction. For germinated and non-germinated peanut, the total phenolics content was also decreased significantly ( $p < 0.05$ ) from  $43.79 \pm 2.381$  to  $18.777 \pm 1.698$  with 57.12% reduction.

**Table 1.** Content of total and non-tannin phenolics and tannin in extracts of germinated and non-germinated soy bean and peanut

Sample	Phenolics content <sup>a</sup>		
	Total	Non-tannin	Tannins
Non-germinated soy bean	13.767±0.294*	8.1±0.7	5.67±0.94
Germinated soy bean	10.673±0.434*	6.6±0.36	4.073±0.39
Non-germinated peanut	43.79±2.381*	11.1±0.655*	32.69±2.392*
Germinated peanut	18.777±1.698*	7.067±0.252*	11.71±1.447*

<sup>a</sup>Expressed as mg gallic acid equivalent/mg dry weight  
Means marked by (\*) are significantly different ( $p < 0.05$ ) between non-germinated and germinated soy bean and peanut.

For tannin content, non-germinated peanut had the highest content of tannin with  $32.69 \pm 2.392$  followed by germinated peanut ( $11.71 \pm 1.447$ ), non-germinated soy bean ( $5.67 \pm 0.94$ ) and germinated soy bean ( $4.073 \pm 0.39$ ). Comparing the germinated and non-germinated soy bean, tannin content was decreased from  $5.67 \pm 0.94$  to  $4.073 \pm 0.39$  with 28.17% reduction. For germinated and non-germinated peanut, tannin content was decreased significantly from  $32.69 \pm 2.392$  to  $11.71 \pm 1.447$  with 64.18% reduction.

It is expected that total phenolics and tannin content changed differently in different legumes (Khandelwal *et al.*, 2010). Khandelwal *et al.* (2010) reported that total phenolics and tannin content was reduced significantly in germinated green gram compared to Bengal gram, red gram and lentil. Loss of total phenolics and tannin contents could be as high as 96% in germinated kidney bean as shown by Shimelis and Rakshit (2007). However, Duenas *et al.* (2009) found that germination increased total phenolics content in lupin seeds after 9 days, the same was reported by Chai (2011) using germinated peanut.

The observed reduction in tannin content after germination was a result of formation of

hydrophobic association of tannins with seed proteins and enzymes. In addition, loss of tannins during germination also may be due to the leaching of tannins into the water (Shimelis and Rakshit, 2007) and binding of polyphenols with other organic substances such as carbohydrate or protein (Saharan *et al.*, 2002). Apart from that, during the period of soaking prior to germination, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols (Saxena *et al.*, 2003; Khandelwal *et al.*, 2010).

### Phytic acid content

Phytic acid content was analyzed in germinated and non-germinated soy bean and peanut and the result was shown in Table 2. From the result obtained, non-germinated soy bean contain the highest content of phytic acid with  $63.358 \pm 0.463$ , followed by germinated soy bean ( $49.584 \pm 1.021$ ), non-germinated peanut ( $21.124 \pm 2.373$ ) and germinated peanut ( $13.074 \pm 1.1$ ). Comparing the result for germinated and non-germinated soy bean, phytic acid content was significantly decreased (21.74%) after germination. Meanwhile, for germinated and non-germinated peanut, phytic acid content was decreased for 38.11% after germination.

**Table 2.** Phytic acid content of germinated and non-germinated soy bean and peanut

Sample	Phytic acid content <sup>a</sup>
Non-germinated soy bean	63.358±0.463*
Germinated soy bean	49.584±1.021*
Non-germinated peanut	21.124±2.373
Germinated peanut	13.074±1.1

<sup>a</sup>Expressed as mg/100 g dry weight  
Means marked by (\*) are significantly different ( $p < 0.05$ ) between non-germinated and germinated soy bean and peanut

The decreased of phytic acid contents of germinated legumes has been frequently reported (Ibrahim *et al.*, 2002; Khattak *et al.*, 2007; Ghavidel and Prakash, 2007). The reduction could be due to increase in endogenous phytase activity (Shimelis and Rakshit, 2007; Khattak *et al.*, 2007) depending on different types of legume. It could also be due to diffusion into the soaking medium also known as leeching out. Soaking of legumes in distilled water was an effective way of removing phytic acid from legumes (Liang *et al.*, 2009).

Phytates play an important role in mineral availability (Phillippy and Johnston, 1985; Khattak *et al.*, 2007; Shimelis and Rakshit, 2007). Phytic acid reduces the availability of zinc, manganese, copper, molybdenum, calcium, magnesium, iron as well as protein (Maga 1982; Beleia *et al.*, 1993). When bound to protein, phytic acid induces a decrease of solubility and functionality of the protein (de Khano and Jost, 1979; El Adawy *et al.*, 2002).

## Conclusions

Germination process was shown to reduce the total phenolic, tannin and phytic acid contents. The reduction of antinutrients may improve the nutritional quality of legumes. From the results, germinated peanut has the biggest reduction in all of the parameters measured compared to germinated soy bean. Germinated beans can be incorporated with wheat-based food product such as bread or pasta to improve nutrition content.

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