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Effect of Processing on Phenolic Content and Antioxidant Activity of Four Commonly Consumed Pulses in China

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Abstract

Four commonly consumed pulses, faba bean, mung bean, soybean, zauki bean were studied for their total phenolic content and antioxidant activity after germination (12 and 24 h) and pressure cooking. Soybean had the highest total phenolic content (6.89 mg ferulic acid/g flour) whereas azuki bean had the least (2.54 mg/g). All pulses, except azuki bean, showed a significant decrease in total phenolic content after germination. The antioxidant activity of the pulses varied from 10.82 to 36.41% (DPPH radical scavenging activity), which significantly decreased with germination in all pulses except azuki. The total phenolic content highly correlated with the antioxidant activity in the pulses. Cooking lowered the total phenolic content by 12-51% and antioxidant activity by 16-67% in the control and germinated pulses.

Keywords: Pulses; Total phenolic content; Antioxidant activity

Introduction

Pulses are well known to be an economical source of protein, carbohydrate and fibre, and are low in fat. Pulses are also incorporated in human diets for their additional nutritional benefits, especially their microconstituents including phenolic compounds, oligosaccharides, enzyme inhibitors, phytosterols and saponins [1-3]. Intake of legumes is reported to potentially lower the risk of cancer, CVD, hypertension and diabetes [4-6]. Some of the microconstituents are currently marketed as functional foods and nutraceutical ingredients [7]. Also, there have been many attempts to incorporate pulses into food products for enrichment of product quality and additional health benefits [8,9].

Sprouting is the practice of soaking, draining and leaving seeds until they germinate and begin to sprout. It has been identified as an inexpensive and effective technology for improving the nutritional quality of cereals and grain legumes. As water is introduced, enzyme inhibitors are disabled and the seed explodes to life [10-13]. As germination proceeds, and enzymes trigger elaborate biochemical changes [14,15]. According to Lorenz [16] the practice of sprouting of cereal grains and legume has become popular in the western world. They can be used in many different foods including breakfast items, salads, soups, casseroles, pasta, and baked products.

The antioxidant capacity of plant foods is derived from the cumulative synergistic action of a wide variety of antioxidants such as vitamins C and E and polyphenols, mainly phenolic acids and flavonoids, carotenoids, terpenoids, Maillard compounds and trace minerals [17]. Polyphenols are probably the most investigated molecules of nutritional interest. Several plant polyphenols are natural antioxidants with an interesting future in various fields such as food and medicine. Because natural antioxidants have shown a reduction in oxidative stress [18], some flavonoids have been assayed in various diseases affecting the heart, brain, and other disorders, including those leading to cancer [19,20].

In China, pulses are the main source of protein and faba bean, mung bean, soybean and zauki bean are commonly consumed pulses. The pulse along with water, salt, and some spice is cooked in a pot or pressure cooker till the grain bursts and a soup like consistency is formed. Pulses are carriers of phenolic compounds and have significant antioxidant potential. The changes occurring in the phenolic content and antioxidant activity upon germination and especially after pressure cooking have not been reported. Given the important role that pulses play in nutrition, the changes occurring in the total phenolic content and antioxidant activity as a result of germination and pressure cooking needed to be investigated.

Materials and Methods

Germination

Faba beans (*Vicia faba* L.) mung bean (*Vigna radiata* L.), soybean (*Glycine max* (L.) Merrill.) and Azuki beans (*Vigna angularis* L.) were collected from local market of the same batch in Nanjing, Jiangsu Province, P.R. China. The pluses were cleaned and steeped for 24 h at 25°C and care was taken that water was changed at 2 h intervals. After soaking, pulses were allowed to germinate in an incubator at 25°C and 100% RH for 12 and 24 h. The germinated pulses were dried in a dryer at 40°C and were ground to pass through a 60 mesh sieve and packed in air tight bags for further analysis.

Cooking

Whole pulses 50 g (control or germinated) were taken in a 3 L capacity Homemaker pressure cooker (Homemaker Cookers Limited, Nanjing, China) with four-fold of water and cooking done at a pressure of 3.3 N/m^2 for optimum time. Preliminary trials were carried out to determine the optimum cooking time. The time at which the pulses split and showed no internal white core when pressed between two glass slides was taken as the cooking time. The cooking time was faba bean (15 min), mung bean (14 min), soybean (12 min), and azuki bean (11 min). The cooked pulses were freeze dried in a freeze dryer. The freeze-dried pulses were ground with a hand grinder and passed through a 60 mesh sieve and packed in air tight bags to prevent any moisture gain till further analysis.

Total Phenolic Content

The total phenolic contents of faba beans (*Vicia faba* L.) mung bean (*Vigna radiata* L.), soybean (*Glycine max* (L.) Merrill.) and Azuki beans (*Vigna angularis* L.) were determined according to Xu and Chang [21] with slight modifications. After adding Folin-Ciocalteau reagent and sodium carbonate to aliquots of samples, the mixtures were set in a 40°C water bath for 20 min. The absorbance was measured at 740 nm using a spectrophotometer (Unico, Shanghai, China) and total phenolic contents were expressed as milligrams of ferulic acid equivalents per grams of defatted sample.

Antioxidant Activity

The antioxidant activity was determined by DPPH assay according to Llorach et al. [22] with some modifications. Aliquot of 200 mL sample mixed with 3.8 mL DPPH solution (200 mM in methanol) was incubated in dark at room temperature for 60 min, then its absorbance at 517 nm was measured by a spectrophotometer. Scavenging ability of the sample to DPPH radical was determined according to the following equation:

Antioxidant activity (AA) was expressed as percentage inhibition of DPPH radical by using below equation;

$$AA=100-[100\times(Asample/Acontrol)]$$

where Asample is the absorbance of the sample at t=60 min, and Acontrol is the absorbance of control.

Statistical Analysis

Data were analysed with SPSS (Statistical Package for the Social Sciences) 13.0 for windows. The mean and standard deviation of means were calculated. The data were analysed by one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate means. Significance was accepted at a probability p<0.05.

Results and Discussion

The ash content of the pulses was insignificantly (p<0.05) affected by germination (Table 1). Faba bean, mung bean, soybean and zauki bean showed no significant change after 24 h of germination. Faba bean showed some decrease, whereas mung bean showed an insignificant increase in the ash content. Akpapunam and Achinewhu [23] observed similar results for ash content in different germinated pulses and legumes.

The protein content in faba bean increased significantly (p<0.05) up to 24 h germination (Table 1). The increase in protein content may be attributed to the synthesis of cell constituents and enzymes, which lead to degradation of other constituents [24]. However, in soybean, the protein content significantly (p<0.05) decreased up to 12 h germination and then further increased upon 24 h germination. The protein content in mung bean significantly (p<0.05) increased up to 12 h germination and then decreased up to 24 h germination.

Variety	Ash (%)			Protein (%)			Fat (%)			Crude fiber (%)		
	Control	12 h	24 h	Control	12 h	24 h	Control	12 h	24 h	Control	12 h	24 h
Faba bean	3.6^a	3.4^a	3.4^a	25.18^a	26.84^b	28.56^c	2.54^a	2.61^a	2.87^b	4.23^a	4.35^a	4.52^b
Mung bean	2.9^b	3.1^a	3.2^a	23.15^a	24.84^b	23.51^a	1.24^a	1.28^a	1.46^b	4.35^a	4.42^a	4.74^b
Soybean	2.0^a	1.7^a	2.0^a	24.35^b	22.16^a	24.37^b	1.56^a	1.61^a	1.86^b	2.84^a	2.94^b	3.04^b
Azuki bean	3.1^a	3.4^a	3.1^a	22.65^a	21.23^a	22.69^a	1.87^a	1.92^a	2.12^b	3.41^a	3.66^b	3.69^c

Table 1: Effect of germination duration on proximate composition of different pulses. a, b and c superscripts are significantly (p<0.05) in different row within a cultivar.

The fat content increased significantly (p<0.05) in all the beans up to 24 h germination (Table 1). All germinated samples contained more ether extractable lipids than the raw, which may be attributed to dissociation of lipid complexes [23]. Lee et al. [25] reported that the crude fat and protein content increased significantly after germination in brown rice. Increase in these constituents has also been reported by Kim et al. [26] for soybeans and by Jung et al. [27] for germinated brown rice.

The crude fiber content significantly (p<0.05) increased in faba bean, mung bean, soybean and zauki bean up to 24 h germination (Table 1). An increase in the dietary fiber after germination has been reported by Lee et al. [25] for brown rice, Lee et al. [28] for buckwheat, and Kim et al. [26] for soybeans.

Effect of Germination on total phenolic content (TPC)

The TPC varied from 3.25-6.89 mg/g in the different pulses with soybean showing the highest content and mung bean showing the least (Table 2). The TPC in faba bean decreased by 43.94% up to 12 h germination and then showed an increase of 20.06% upon further 12 h germination.

In mung bean, the TPC significantly (p<0.05) decreased by 16.62% upon 12 h germination and further decreased by 46.49% upon 24 h germination. Randhir et al. [29] reported that germination causes a decrease of total phenolic content in Green mung. Barroga et al. [30] reported similar total phenolic content values for raw and 24 h germinated Mung bean.

In azuki bean, the TPC significantly (p<0.05) increased by 52.75% in the first 12 h of germination and showed a significant increase of

27.84% upon germination for 24 h. Tian et al. [31] reported that during germination, the bound phenolic compounds become free and lead to an increase in the total phenolic content.

Sample	Germination time (h)	Total phenolic content (mg/g)	Antioxidant activity (%)
Faba bean	Control	5.78 ± 0.54^{b}	36.24 ± 0.47^{c}
	12	3.24 ± 0.21^{a}	16.34 ± 0.23^{a}
	24	3.89 ± 0.34^{a}	20.26 ± 0.14^{b}
Mung bean	Control	3.25 ± 0.31^{c}	16.24 ± 0.26^{c}
	12	2.71 ± 0.21^{b}	13.65 ± 0.18^{b}
	24	1.45 ± 0.14^{a}	10.56 ± 0.16^{a}
Soybean	Control	6.89 ± 0.48^{c}	36.41 ± 0.64^{b}
	12	5.27 ± 0.45^{b}	21.33 ± 0.54^{a}
	24	4.13 ± 0.36^{a}	19.88 ± 0.26^{a}
Azuki bean	Control	2.54 ± 0.47^{a}	10.82 ± 0.54^{a}
	12	3.88 ± 0.36^{b}	13.41 ± 0.76^{b}
	24	4.96 ± 0.24^{c}	17.56 ± 0.88^{c}

Table 2: Effect of germination on total phenolic content and antioxidant activity of pulses. a, b and c superscripts are significantly (p<0.05) in different cloumn within a cultivar.

Fernandez-Orozco et al. [32] reported that the total phenolic content significantly decreased after 2 days of germination but then increased as germination time increased to 4 days. During germination, the endogenous enzymes of the legumes are activated and the most important enzymes are the hydrolases and polyphenoloxydases, whose activity increases during germination depending on the type of legume. Khattak et al. [33] reported that germination time up to 48 h significantly reduced the phytic acid content in chickpea.

Effect of Germination on Antioxidant Activity

The antioxidant activity in control samples ranged from 10.82-36.41% with the highest activity exhibited by soybean and the lowest exhibited by azuki bean (Table 2). Antioxidant activity is expressed as percent DPPH radical scavenging activity with higher values indicating greater antioxidant activity.

During germination of faba bean, antioxidant activity significantly decreased (p<0.05) by 54.91% at 12 h germination and as germination increased from 12 to 24 h, it significantly increased by 23.99%. The total phenolic content and antioxidant activity for faba bean showed a positive correlation coefficient of 0.98.

In mung bean, the antioxidant activity insignificantly (p<0.05) decreased by 15.95% after 12 h germination and after 24 h germination, further significantly decreased by 22.64%. The total phenolic content and antioxidant activity showed a positive correlation coefficient of 0.99. Fernandez-Orozco et al. [32] reported that as compared to raw seeds, sprouts of mung bean and soybean had more total phenolic compounds, and germination is a good process for obtaining functional flours with greater antioxidant capacity and more antioxidant compounds than the raw legumes.

The antioxidant activity in soybean significantly (p<0.05) decreased by 41.41% after 12 h germination; after germination for 24 h, it showed a insignificant decrease of 3.61%. Soybean showed a positive correlation coefficient of 0.98 between the total phenolic content and antioxidant activity. Oboh [34] also studied the antioxidant activity of legumes and found a positive correlation between phenolic compounds and antioxidant activity.

The antioxidant activity in azuki bean significantly (p<0.05) increased by 23.94% after germination for 12 h and further increased by 30.94% as germination time increased to 24 h. The total phenolic content and antioxidant activity showed a positive correlation coefficient of 0.99 in azuki bean. Correlation between the total phenolic content and antioxidant activity of some plant foods has been reported by Sun et al. [35], Chu et al. [36], and Yang et al. [37].

Effect of Cooking on TPC

The total phenolic content after cooking (Table 3) significantly (p<0.05) decreased by 27.35 and 29.12% in the control and 12 h germinated faba bean and further decreased by 1.42% after 24 h germination but this decrease was insignificant. Rocha-Guzman et al. [38] studied three common bean cultivars for phenolic content and free radical scavenging activity before and after autoclaving and reported that phenolic content in common beans after pressure cooking was reduced by 90%. Barroga et al. [30] found that boiling and cooking reduced the amount of phenols in legumes by 73%.

Treatment	Variety			
	Faba bean	Mung bean	Soybean	Azuki bean
Control	5.85 ± 0.54^{b}	3.24 ± 0.21^{b}	7.24 ± 0.56^{b}	6.54 ± 0.56^{a}
Cooked	4.25 ± 0.41^{a}	2.86 ± 0.23^{a}	4.68 ± 0.35^{a}	3.21 ± 0.32^{b}
Control (12 h)	3.88 ± 0.36^{b}	2.97 ± 0.34^{b}	5.46 ± 0.74^{b}	3.35 ± 0.41^{a}
Cooked	2.75 ± 0.28^{a}	2.34 ± 0.29^{a}	3.68 ± 0.36^{a}	2.85 ± 0.24^{b}
Control (24 h)	4.21 ± 0.24^{a}	2.41 ± 0.26^{a}	4.34 ± 0.41^{b}	3.48 ± 0.35^{a}
Cooked	4.15 ± 0.31^{a}	2.16 ± 0.27^{b}	3.14 ± 0.36^{a}	2.11 ± 0.24^{b}

Table 3: Effect of cooking on total phenolic content in control, 12 h and 24 h germinated pulses. a and b superscripts are significantly (p<0.05) in different column within a cultivar.

In mung bean, the total phenolic content in control significantly (p<0.05) decreased by 11.73% after cooking, whereas in 12 and 24 h germinated samples it significantly decreased by 21.21 and 10.37%, respectively (Table 3).

After cooking the control, 12 and 24 h germinated soybean in the pressure cooker, the TPC significantly decreased by 35.36, 32.60, and 27.65%, respectively (Table 3).

In azuki bean, the total phenolic content after cooking significantly (p<0.05) decreased by 50.92, 14.92, and 39.37% in the control, 12 and 24 h germinated samples (Table 3). Vidal-Valverde et al. [39] and Rocha-Guzman et al. [38] reported similar results for cooked beans and observed a significant decrease in total polyphenols for soaked and germinated Masur.

Effect of cooking on antioxidant activity

After cooking, the antioxidant activity significantly ($p<0.05$) decreased (Table 4) by 54.91, 52.42, and 27.50% in the control, 12 and 24 h germinated samples. Faba bean showed a positive correlation coefficient of 0.99 between the total phenolic content and antioxidant activity.

In mung bean, cooking significantly lowered the antioxidant activity ($p<0.05$) by 15.66, 43.25, and 32.31% in the control, 12 and 24 h germinated samples (Table 4). The total phenolic content and antioxidant activity showed a correlation coefficient of 0.88 in mung bean.

Treatment	Variety			
	Faba bean	Mung bean	Soybean	Azuki bean
Control	36.17 ± 2.34^b	14.88 ± 2.12^b	35.16 ± 2.65^b	38.59 ± 3.65^b
Cooked	16.31 ± 2.11^a	12.55 ± 1.56^a	14.25 ± 1.36^a	12.65 ± 1.35^a
Control (12 h)	15.89 ± 1.56^b	14.89 ± 1.35^b	16.35 ± 1.56^b	18.69 ± 1.88^b
Cooked	7.56 ± 0.89^a	8.45 ± 0.95^a	7.55 ± 0.94^a	11.44 ± 1.85^a
Control (24 h)	18.69 ± 1.45^b	12.69 ± 1.56^b	17.45 ± 2.14^b	17.69 ± 2.14^b
Cooked	13.55 ± 1.22^a	8.59 ± 0.88^a	8.23 ± 0.96^a	8.41 ± 0.98^a

Table 4: Effect of cooking on antioxidant activityt in control, 12 h and 24 h germinated pulses. a and b superscripts are significantly ($p<0.05$) in different column within a cultivar.

In soybean, the antioxidant activity significantly ($p<0.05$) decreased after cooking by 59.47, 53.82, and 52.84% in the control, 12 and 24 h germinated samples (Table 4). Soybean showed a positive correlation coefficient of 0.97 for the total phenolic content and antioxidant activity.

Cooking lowered the antioxidant activity significantly ($p<0.05$) by 67.22, 38.79, and 52.46% in the control, 12 and 24 h germinated azuki bean samples (Table 4). Azuki bean showed a positive correlation coefficient of 0.95 for the total phenolic content and antioxidant activity.

Conclusions

It was concluded that germination lowered the phenolic content and antioxidant activity in all pulses except azuki bean in which the TPC and antioxidant activity increased with germination. Cooking significantly lowered the total phenolic content and antioxidant activity in the pulses. There was a significant correlation between the total phenolic content and antioxidant activity both in germinated and cooked pulses.

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