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RESEARCH ARTICLE

Impact of soaking and germination procedures on polyphenols, tannins, and phytate contents in some Egyptian pulses

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Abstract

Whereas the nutritional value of pulses is generally recognized, the presence of antinutritional elements in their composition limits their use. Effect of soaking and germination process on removal or reducing of (total phenolic, tannins and phytic acid) content of commonly consumed pulses in Egypt were studied. Four pulses namely faba bean *(Vicia Faba)* Giza 843, chickpea *(Cicer arietinum)* Giza 1, cowpea *(Vigna unguiculata)* krymy 7 and soybean *(Glycine max)* Giza 111 were used in this research. The total phenolic compounds, tannins, and phytic acid content of four pulses were significantly decreased after soaking for 12 hours and germination treatment for varied periods (24, 48, and 72 hours). On a dry weight basis, the phenolic compounds content of raw pulses was 370.9, 132.5, 763.4, and 249.4 mg/100g, for faba bean, chickpea, cowpea, and soybean respectively, while tannin content was 684.5, 488.1, 390.9, and 225.5 mg/100g, and phytic acid content was 1050.6, 719.2, 987.2, and 1076.2 mg/100g. Soaking for 12 hours significantly decreased the concentration of total phenolics, tannins and phytic acid contents of the investigated pulses by 4.0-22.7%, 7.1-26.5% and 7.0-15%, respectively. Germination process for 72 hours reduced total phenolics, tannins and phytic acid contents of studied pulses by 21.4 -56.9%, 23.9-64.8% and 54.6-65.0%, respectively. From the obtained results it could be concluded that the reduction of antinutritional factors content was increased with the progress of both soaking and germination periods in all studied pulses.

Keywords: Germination; Phytic acid; Pulses; Soaking; Tannins; Total phenolic.

1. Introduction

Pulses are one of most important food sources in the world's. It has a significant impact on human nutrition. Which are consumed in large quantities in Middle East countries, and contribute as a major source of important nutrients for many people in developing countries (Negi *et al.,* 2001; Kamchan *et al.,* 2004). On a worldwide scale, pulses seeds provide one-fifth of all plant proteins ingested by people. Pulses are also a cheap and beneficial prospective supply of high-quality protein, notably for the impoverished Egyptians and vegetarian population. Pulses are also a cost-effective source of supplemental proteins for a huge population in developing nations. Pulses have long been known as functional foods that promote good health and provide medicinal capabilities, in addition to their nutritional benefits. Pulses, also had low glycemic index for diabetes patients (Chillo *et al.,* 2008), breast cancer preventive measures (Adebamowo *et al.,* 2005), and cancer health promotion and health benefits with regard to cardiovascular disease (Goni and Valentin-Gamazo, 2003) and bone strength (Alekel *et al.,* 2000). In Egypt, pulses such as faba bean, soybean, chickpea and cowpea are widely consumed because of their nutritional quality (Mittal *et al.,* 2012). These pulses are also low-cost and high in-complex carbohydrates, protein, soluble fiber, vitamins, and minerals. (Ibrahim *et al.,* 2002; Costa *et al.,* 2006). Unfortunately, pulses contain considerable amounts of bioactive chemicals with toxic and/or antinutritional characteristics that might disrupt consumers' body metabolism and negatively affect nutritional quality (Dragievi *et al.,* 2010). Malnutrition is a result of these substances, which primarily affects people in developing nations (Kumari *et al.,* 2014). The

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major groups of antinutritional factors present in pulses are trybsin inhibitors, phytic acid, tannin, phenols, etc which may have adverse effects for human nutrition. Pulses contain a variety of antinutritional factors which directly or indirectly interfere with their nutritional quality (Martı´n-Cabrejas *et al.,* 2009; Kumar *et al.,* 2010). The presence of polyphenols produces a sensation of astringency. The precipitation of salivary proteins and muco polysaccharides is assumed to be the cause. Polyphenols in the gastrointestinal tract have a number of negative effects, including iron absorption suppression, esophageal cancer (Baxter *et al.,* 1997), and irreparable complex formation of gut enzymes and food proteins (Baxter *et al.,* 1997; Robbins *et al.,* 1991). Tannins are polyphenolic compounds with varying molecular weights and degrees of complexity they are a group of chemically undefined chemicals that have the ability to occur protein in aqueous solution (Makkar, 2003). In addition, phytic acid makes very stable compounds with minerals, making them inaccessible for intestinal absorption or metabolism. Phytate chelates a variety of metal ions, preventing them from being absorbed. Phytic acid, often known as phytate, is the most common type of phosphorus storage in plant tissues (Kumar *et al.,* 2010). It can also link to starch both directly and indirectly through proteins by hydrogen bonding with a phosphate group. Phytate hydrolysis is caused by either phytase or non-enzymatic cleavage. Enzymes that hydrolyze phytate are found in a diverse range of microorganisms, plants, and mammals (Urbano *et al.,* 2000). Effective and cheap processing techniques like soaking and germination were widely regarded as effective strategies for lowering the levels of antinutritional substances in numerous legume seeds, which was necessary to increase the nutritional content of pulses. Treatment processes can improve the nutritional value of pulses, allowing raw grains to be transformed into useful products with the highest nutritious value, ensuring population nutrient security in developing nations. The goal of this research was to study the effect of both soaking and germination procedures on the polyphenols, tannins, and phytate levels of certain common Egyptian pulses.

2. Materials and Methods

2.1. Materials

Faba bean (*Vicia Faba*) seeds Giza 843, chickpea (*Cicer arietinum*) seeds Giza 1, cowpea (*Vigna unguiculata*) seeds Krymy 7, and soybean (Glycine max) seeds Giza 111. During the 2014 season, they were collected from the Agronomy Research Institute (Shandaweel Agricultural Research Center, Sohag, Egypt).

2.2. Chemicals

All chemicals used in this study were purchased from Pio Chem Company and Sigma – Aldrich.

2.3. Preparation of samples

2.3.1. Raw seeds

Whole dry pulses were manually cleaned of broken seeds, dust, and other foreign materials before being processed into fine flour in a lab grinder (Braun grinder, ZK 500). The ground samples were stored at 4°C until they were analyzed.

2.3.2. Soaking

A sample of cleaned seeds was soaked in distilled water at room temperature for 12 hrs (water was changed every 6 h). Seed to water ratio was 1:5 (w/v). The soaking water was thrown and the soaked seeds were washed twice with ordinary water followed by rinsing with distilled water .The soaked seeds were dried in a hot air oven at (60-70)ºC to a constant weight and milled with a laboratory grinder (Braun grinder, ZK 500) to obtain fine flour. The ground samples were kept at 4°C until analysis (Abdel-Gawad, 1993; Sorour, 2002; Afify *et al.,* 2011).

2.3.3. Germination

A portion of the soaked seeds (12 hrs) was germinated on wet filter paper for 24, 48, and 72 hrs at room temperature, with frequent seeds watering with sterilized water every 12 hours. To prevent microbial growth, the seeds were washed with a 0.3 percent sodium hypochlorite solution every 12 hrs. The germinated seeds were then dried in a hot air oven at 60 °C for 36 hours to a constant weight, and then ground in an electric grinder. The powder

samples were kept in sealed bottles at 4°C until they were analyzed (Abdel-Gawad, 1991; Cevallos-casals and Cisneros- Zevallos, 2010).

2.4. Analytical methods

2.4.1. Gross chemical composition

Moisture, crude oil, crude protein, crude fiber and ash contents were determined according to A.O.A.C. standard methods (AOAC, 2019). Total carbohydrate content of samples was calculated by difference.

2.4.2. Determination of total phenolic compounds

Total phenolics were determined using the Folin-Ciocalteau method with slight modification (Taga *et al.*, 1984). The extracts of samples and standards were prepared in (0.3% acidified methanol water 60:40). The100µl of test solutions were added to 2.0 ml of 2% $Na2CO_3$. After 2 min, 50 µl of 50% Folin-Ciocalteau reagent was added. The mixture was incubated at 37°C for 30 min in the dark. The absorbance was then measured at 750 nm on a spectrophotometer (Uviline 9400, Schoott Instrument-EU) against the blank that consisting of all reagents and solvents without test compounds. The phenolic concentrations were determined by comparison with the standard calibration curve and specified as mg TPC/100g extract given as Gallic Acid Equivalents (GAE).

2.5. Determination of Tannins

Quantitative estimation of tannins was carried out using the modified vanillin–HCl method (Price *et al.*, 1978) as described by (Babiker and El-Tinay, 1992). Two grams of each sample was extracted with 50 ml absolute methanol for 20 minutes at room temperature with constant agitation. After centrifugation for 10 minutes at 653 r.p.m., 5 ml of Vanillin-HCl reagent was added to the extract (1 mL) and the colour developed after 20 min at room temperature was read at 500 nm. A standard curve was prepared using catechin (Sigma Chem. CO., St. Louis, Mo) after correcting for blank, tannins concentration was expressed in mg catechin equivalents.

2.6. Determination of Phytate Content

Phytic acid content in legume pulses samples was extracted and estimated according to the procedure described method in (AOAC, 2019). Dowex® 50WX8 hydrogen form hydrogen form, 200-400 mesh, and phytic acid was determined from the standard curve according to the equation: Phytic acid (mg / 100g dw) = Phytate P × 3.546.

2.7. Statistical analysis

Data were statistically analyzed using analysis of variance and least significant difference (LSD) using (version 9.1, SAS Institute 2003). Significant differences were determined at the 5% level of significance.

3. Results and Discussion

3.1. Chemical composition

Gross chemical compositions of raw studied pulses (faba bean, chick pea, cowpea and soybean) were showed in Table 1. The data revealed that soybean has the highest level of protein content, while chickpea recorded the lowest value. The values of protein were: 29.38, 20.72, 26.30 and 39.56% on dry weight basis in raw faba bean, chick pea, cowpea and soybean, respectively. On a dry weight basis, the crude fat content of faba bean, chick pea, cowpea, and soybean was 1.26, 6.45, 3.16, and 22.33 percent, respectively. In comparison to the other investigated pulses, the soybean had the highest level of ash (5.32%), while the chickpea had the lowest (3.07%). , according to the results in the same table , the crude fiber content of faba bean seeds was higher (6.93%) than that of other samples. On the other hand the pulses are considered important source of carbohydrates. Carbohydrate levels were 58.56, 66.35, 63.84 and 27.86 % in faba bean, chick pea, cowpea and soybean, respectively. These results are in agreement with those reported by Alajaji and El-Adawy (2006), Iqbal *et al.* (2006), Kayembe (2011) and Desalegn (2015).

Table 1. Gross chemical composition of pulses (% on dry weight basis)*.

Samples	Protein	Fats	Fiber	Ash	Carbohydrate**
Faba bean	29.38	1.26	6.93	3.87	58.56
Chickpea	20.72	6.45	3.41	3.07	66.35
Cowpea	26.3	3.16	2.99	3.71	63.84
Soybean	39.56	22.33	4.93	5.32	27.86

*Average of three replicates. ** Calculated by difference.

3.2. Total phenolic content

The results presented in Table (2) showed that the effect of soaking on phenolic contents in faba bean, chick pea, cowpea and soybean. Cowpea has higher phenolic content than other studied pulses. Raw faba bean, chick pea, cowpea, and soybean samples had phenolic levels of 370.95, 132.59, 763.42, and 249.40 mg GAE/100g, respectively. These findings are consistent with Mondor *et al.* (2009), Malencic´ *et al.* (2012) and Coda *et al.* (2015). Soaking of pulses in distilled water for 12 hours lowered phenolic compounds content compared with control. Soaking processes showed a significant decrease in phenolic content of pulses. The percentages of losses were, 3.9%, 15.9%, 22.7% and 8.2% of its initial values of control in faba bean, chick pea, cowpea and soybean, respectively. Preet and Punia (2000) and Alonso *et al.* (2000) reported similar results for cowpeas after soaking. The decrease in polyphenol concentrations caused by soaking has been attributed to many factors. According to Igbedioh *et al.* (1995) and Khandelwal *et al.* (2010), the phenolic content reduction could be due to simple leaching into the soak water. Reduced extractability, which occurs when lower molecular weight phenolic substances polymerize and become insoluble in water, may also be to blame for losses. Bravo (1998) and Saharan *et al.* (2002) have attributed the losses to binding of polyphenols with other organic substances such as carbohydrates or protein. Alternatively, during the period of soaking, the enzyme polyphenoloxidase may be activated, resulting in degradation and consequent losses of polyphenols (Jood *et al.*, 1998; Saxena *et al.*, 2003). Considering polyphenols are present in the seed's peripheral, there is a chance t they will make their way into the soaking medium via the seed coat (Afify *et al.*, 2012).

Table 2. Effect of soaking and germination process on phenolic content in pulses (mg GAE/100g dw).

Samples	Control	Soaking	Germination			L.S.D 5%
		12 h	24 h	48 h	72 h	
Faba baen	$370.95^{a}\pm0.82$	$356.55^{b}\pm0.56$	$335.99^{c}\pm1.33$	$304.67^{d}\pm0.84$	$291.51^{e}\pm0.87$	1.901
Chickpea	$132.59^{a}\pm0.86$	$111.53^{b}\pm0.61$	$96.89^{c}\pm0.80$	$82.98^{d}\pm0.86$	$75.55^{e}\pm0.50$	1.5246
Cowpea	$763.42^{a}\pm1.66$	$589.95^{b}\pm1.07$	$454.63^{c}\pm0.84$	$385.44^{d}\pm0.46$	$328.55^{e}\pm0.51$	2.0224
Soybean	$249.40^{a}\pm0.83$	$228.91^{b}\pm0.89$	$210.28^{c}\pm0.81$	$199.20^{d}\pm0.84$	$186.35^{e}\pm0.86$	1.5444

Means± standard deviation (SD) on dry weight basis.

$^{a-e}$Values in the same row with different superscript letters differ significantly at 5% level of significance.

Germination of pulses led to a significant decrease in polyphenol content, the reduction was greater when germination was carried out for a longer time (Table 2). Phenolic content in pulses was decreased gradually during germination period. After 24, 48, and 72 hours of germination, phenolic compounds in faba bean were reduced by 9.4, 17.8, and 21.4 percent of their initial levels, respectively. In chick pea phenolic content decreased after the same periods of germination by 26.9, 37.4 and 43% of its initial value, respectively. While, these decrements were about 40.4, 49.5, and 56.9% for cowpea compared with control after the same periods of germination, respectively. After the same germination times, phenolic content values for soybean were 15.6, 20.1, and 25.2 percent of

their raw legume values, respectively. These results are in agreement with those mentioned by Preet and Punia (2000) and Alonso *et al.* (2000). The reduction in phenolic compounds content during germination may have been a function of the presence of polyphenol oxidase and enzymatic hydrolysis (Jood *et al.*, 1987, Paramjyothi and Mulimani, 1996). Leaching of polyphenols into the soaking water prior to germination may also partly account for losses.

3.3. Tannins

Data in Table (3) shows the effect of soaking and germination on tannins contents in faba bean, chick pea, cowpea and soybean. Faba bean has higher tannin content than other studied pulses. Tannin contents in faba bean, chick pea, and cowpea and soybean raw samples were 684.5, 488.1, 390.9 and 225.5 mg catechin equivalent (CE) /100g, respectively. Similar results were given by EL-Adawy (2002), Kalpanadevi and Mohan (2013) and Kumari *et al.* (2014).

3.4. Soaking

Soaking of pulses in distilled water for 12 hours lowered tannin compared with control. Tannin content was decreased by 13.3, 7.1, 26.4 and 13.4 % of its initial values of control in faba bean, chick pea, cowpea and soybean, respectively. These results are in the line with those mentioned by Kalpanadevi and Mohan (2013) and Kumari *et al.* (2014). Since these components, in addition to their predominance in husks (Reddy and Pierson, 1994), are water soluble (Kumar *et al.*, 1979), and so leach into the liquid media, the loss in tannin content after soaking was significantly greater. The presence of water soluble tannins in the soaking medium, or their breakdown by enzymes during the soaking period, could possibly account for losses (Desphande and Cheryan, 1983; Afify *et al.*, 2012).

3.5. Germination

The results given in Table (3) showed the effect of germination process on tannin content in pulses. A significant reduction was observed in the tannin content of pulses due to germination. The levels of reduction in faba bean were 22.5, 26.7 and 28.9 % of its initial values of control after 24, 48 and 72 h of germination, respectively. In chick pea tannin content decreased by 13.3, 18.7 and 23.9% of its control value after the same periods of germination, respectively. This decrement of tannin content was about 48.4, 61.7, and 64.8% for cowpea compared with control after 24, 48 and 72 hours of germination, respectively. While in Soybean it was 24.9, 36.9 and 47.9% of its values in the raw seeds after the same germination periods, respectively. Similar results were given by EL-Adawy (2002), Kalpanadevi and Mohan (2013) and Kumari *et al.* (2014). Increased activity of polyphenol oxidase and other catabolic enzymes may be responsible for the reduction in tannin content of pulses after germination. Activated enzymes cause the hydrolysis of different components during germination. Enzymatic hydrolysis could be blame for the decrement in tannin content following germination (Deshpande *et al.*, 1986; Khandelwal *et al.*, 2010).

3.6. Phytic acid

The effect of soaking and germination on phytic acid content in pulses are shown in Table (4) . In raw samples, faba bean has higher phytic acid content than other investigated pulses. The phytic acid content was 1050.6, 719.2, 987.2 and 1076.2 mg/100g for raw faba bean, chickpea, cow pea and soybean, respectively. These results are in the same line with those reported by Khattab and Arntfield (2009), Karkle1e and Beleia (2010). Results revealed that soaking for 12 h could lower the level of phytic acid content below the control value. The reduction in phytic acid content was 9.3, 7.0, 14.4 and 9.0% for faba bean, chick pea, cowpea and soybean, respectively of the control.

Table 3. Effect of soaking and germination process on tannin content in pulses (mg CE/100g dw).

Treatment	Control	Soaking	Germination			L.S.D 5%
			12 h	24 h	72 h	
Faba baen	684.55^{a}±0.27	593.41^{b}±0.35	530.19^{c}±0.60	501.29^{d}±0.42	486.38^{e}±0.19	0.7202
Chickpea	488.12 ^{a}±0.10	453.22^{b}±0.37	423.06^{c}±0.56	396.79^{d}±0.46	371.34^{e}±0.92	0.9127
Cowpea	390.93^{a}±0.41	287.48^{b}±0.28	201.40^{c}±0.12	149.34^{d}±0.52	137.43^{e}±0.14	0.4525
Soybean	225.50^{a}±0.28	195.21^{b}±0.82	169.29^{c}±0.25	142.17^{d}±0.99	117.47^{e}±0.65	1.3155

Means± standard deviation (SD) on dry weight basis.

$^{a-e}$ Values in the same row with different superscript letters differ significantly at 5% level of significance.

The results approved by Sorour (2002), Ali (2008) and Karkle1e and Beleia (2010). It is possible that soaking reduces phytate levels in pulses since phytic acid in dried beans occurs entirely as a water-soluble salt. Because of the influence of the concentration gradient, the drop in phytate after soaking of pulses could have been due to leaching of phytate ions into the soaking water. The activation of the endogenous phytase during the 12 hour soaking period, as well as product diffusion, could account for some of the losses. As a result of soaking in pulses, there is an increase in phytase activity and a decrease in phytate levels (Kataria *et al.*, 1989). As soaking, germination also resulted in a significant loss of phytic acid in pulses, Table 4. Longer the period of germination, lead to a greater loss in phytic acid. Also, germination reduced phytic acid content and the reduction level increased, as the germination time increased from 24 to 72 h. The levels of phytate reduction in faba bean were 47.2, 57.4 and 63.4 % of its initial values of control after 24, 48 and 72 h of germination, respectively. In chick pea phytic acid content decreased by 39.9, 52 and 60.6 % of its control value after the same germination periods, respectively. While, it was about 37.8, 47.7 and 54.6 % for cowpea compared with control after germination periods, respectively. While in soybean the phytic acid content decreased by 45.6, 56.9 and 65 % of its values in the raw seeds after the same germination periods, respectively. These findings are consistent with those reported by Ali (2008). The decrease in phytic acid in pulses revealed that an increase in phytate hydrolysis during germination resulted in the liberation of inorganic phosphates from organic phosphorus-containing compounds for plant growth (phytate). Phytic acid degradation during germination could be related to an increase in endogenous phytase activity for its usage as a source of inorganic phosphate during germination (Tabekhia and Luh 1980; Eskin and Wiebe, 1983; Egli *et al.*, 2002). Since phytic acid has been considered to be one of the factors responsible for reducing minerals bioavailability, its reduction during germination may enhance the nutritional quality of beans. Because phytic acid is thought to be one of the reasons that reduce mineral bioavailability, decreasing it during germination could improve the nutritional quality of beans.

Table 4. Effect of soaking and germination process on phytic acid content in pulses (mg /100g dw) .

Treatment	Control	Soaking	Germination			L.S.D 5%
			12 h	24 h	72 h	
Faba baen	1050.65^{a}±0.90	952.36^{b}±0.04	554.18^{c}±0.42	447.42^{d}±0.40	383.61^{e}±0.57	0.9987
Chickpea	719.26^{a}±0.35	668.66^{b}±1.17	431.79^{c}±0.55	344.67^{d}±0.46	282.78^{e}±0.55	1.159
Cowpea	987.28^{a}±0.59	844.55^{b}±0.82	613.67^{c}±0.24	515.42^{d}±0.41	448.18^{e}±0.36	1.0633
Soybean	1076.21^{a}±0.10	979.03^{b}±0.30	584.50^{c}±1.12	463.36^{d} ±0.70	376.40^{e}±0.49	0.9776

Means± standard deviation (SD) on dry weight basis..

$^{a-e}$Values in the same row with different superscript letters differ significantly at 5% level of significance.

4. Conclusions

Soaking (12 h) and germination for (72 h) were required to cause a considerable reduction in phenolic compounds, tannins and phytic acid levels in all studied pulses.. These procedures are simple, low-cost in terms of time, energy, and fuel, and may be used to process different pulses at home. Furthermore, they were critical to increase legume nutrient value and fully harness their potential as a human diet.

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