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Polyphenols, Tannins and Phytate Contents in Some Egyptian Legumes as Affected by Soaking and Germination Processes

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Abstract

Nutritional value of pulses is a widely accepted but the presence of anti nutritional factors in its composition imposes a restriction in its consumption. Soaking and germination process on the removal or reducing of (total phenolic, tannins and phytic acid) of commonly consumed legumes in Egypt were studied. Four legumes namely faba bean *(Vicia Faba)* Giza 843, chickpea *(Cicer arietinum)* Giza 1, cowpea *(Vigna unguiculata)* krymy 7 and soybean *(Glycine max)* Giza 111, were investigated. Soaking for 12 hours and germination for different time periods (24, 48 and 72 h) contributed significantly reducing in the total phenolic compounds, tannins and phytic acid content of legumes. Phenolics compounds content of raw legumes were, 370.9, 132.5, 763.4 and 249.4 mg/100g, while, tannin contents were, 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 on dry weight basis in faba bean, chickpea, cowpea and soybean, respectively. Soaking for 12 hours significantly decreased the concentration of total phenolics, tannins and phytic acid contents of the investigated legumes 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 legumes by 21.4-56.9, 23.9-64.8 and 54.6-65.0%, respectively. From the obtained results it could be observed that the removal extent of anti nutritional factors was increased with the progress of germination periods in all studied legumes.

Keywords: Legumes; Soaking: Germination; Total Phenolic; Tannins; Phytic Acid

Introduction

Legumes are one of the most important sources of food in the world. Its plays an important role in human nutrition. Which are consumed in large quantities in Middle East countries, and contribute a lot for diet and also a major source of important nutrients for many people in developing countries [1,2]. Seed of legumes provide one-fifth of all plant proteins consumed by human on a global basis. Legumes also are a cheap and valuable potential source of good-quality protein, in particularly for poor people and vegetarian of the Egyptian population. Legumes also serve as an economical source of supplementary proteins for a large human population in developing countries. In addition to their nutritional value, it has long been recognized that legumes are functional foods that both promote good health and have therapeutic properties, Legumes have been shown to have low glycemic indexes for people with diabetes [3], breast cancer prevention [4], cancer prevention and health benefits with respect to cardiovascular disease due to their dietary fiber content [5] and bone health [6]. In Egypt, legumes such as faba bean, soybean, chickpea and cowpea are consumed widely because of their nutritional quality [7]. These legumes are also inexpensive and rich sources of complex carbohydrates, protein, dietary fibers, vitamins and some minerals including trace elements [8,9]. However, this legumes contain significant amounts of bioactive compounds with toxic and/or anti nutritional properties that can alter the body metabolism of consumers and exert a negative impact on the nutritional quality [10]. This compound lead to malnutrition situations, afflicting human populations mainly in the developing countries [11]. The major group of anti nutritional factors present in legumes are trybsin inhibitors, phytic acid, tannin, phenols, etc which may have adverse effects for human nutrition. Legumes contain a variety of anti nutritional factors which directly or indirectly interfere with their nutritional quality [12,13].

The presence of poly phenols produces a sensation of astringency. This is thought to arise from the precipitation of oral proteins and muco polysaccharides. Poly phenols in the digestive tract display many detrimental effects, including the inhibition of iron absorption, esophageal cancer [14] and irreversible complexation

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of gut enzymes and dietary proteins [15]. Tannins are poly phenolic substances with various molecular weights and a variable complexity, these are chemically not well-defined substances but rather a group of substances with ability to bind protein in aqueous solution [16].

Phytic acid also forms very stable complexes with minerals, thereby rendering them unavailable for intestinal uptake or metabolism. Phytate chelates various metal ions, preventing their absorption. Phytic acid or phytate is the principal storage form of phosphorus in plant tissues [13]. It can also bind with starch directly by hydrogen bonding with a phosphate group and indirectly through the proteins**.** The hydrolysis of phytate occurs as a result of phytase or non enzymatic cleavage. Enzymes capable of hydrolyzing phytate are widely distributed in micro organisms, plants and animals [17].

It was widely accepted that simple and inexpensive processing techniques such as soaking and germination were effective methods for reducing the levels of the anti nutritional compounds in several legume seeds, which was essential to improve the nutritional quality of legumes. Nutritional quality of legumes can be enhanced by processing technologies should help to transform raw grains into useful products with maximum nutritional value to ensure nutrient security of population for developing countries. The improving of the nutritive value of legumes, preparation techniques have been developed to significantly raise the bioavailability of nutrients [18,19].The purpose of this study was an attempt to evaluate the effect of soaking and germination on poly phenols, tannins and phytate content of some common Egyptian legumes.

Materials and Methods

Materials

The seeds of faba bean *(Vicia Faba)* Giza 843, chickpea *(Cicer arietinum)* Giza 1, cowpea *(Vigna unguiculata)* Krymy7, soybean *(Glycine max)* Giza 111. Were obtained from Agronomy Research institute (Shandaweel Agricultural Research Center, Sohag-Egypt) during the season of 2014.

Chemicals

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

Preparation of Samples

Raw Seeds: Whole dry legumes manually cleaned from broken seeds, dust and other foreign materials and ground in a laboratory grinder (Braun grinder, ZK 500) to obtain fine flour. The ground samples were kept at 4°C until analysis.

Soaking: Sample of clean seeds were soaked in distilled water for 12 hours at room temperature (water was changed every 6 h). A seeds / water ratio of 1:5 (w/v) was used. After soaking, the unimbibed water was discarded. The soaked seeds were washed twice with ordinary water followed by rinsing with distilled water then dried in a hot air oven at (60-70)°C to a constant weight and milled in an a laboratory grinder (Braun grinder, ZK 500) to obtain fine flour. The ground samples were kept at 4°C until analysis [20,21,22].

Germination: Part of the soaked seeds (12 hours) were germinated with wet filter paper for 24, 48 and 72 h at room temperature, with frequent watering, the seeds were sprayed every 12 h with sterilized water. The seeds were rinsed with 0.3% sodium hypochlorite solution each 12 hours to inhibit microbial growth. The germinated seeds were then dried at 60°C for 36 hours into a hot air oven to a constant weight and then ground in an electric grinder. The ground samples were kept in closed bottles and stored in a refrigerator at 4°C until analysis [23,24].

Analytical Methods

Gross Chemical Composition: Moisture, crude oil, crude protein, crude fiber and ash contents were determined according to A.O.A.C. standard methods [25]. Total carbohydrate content of samples was calculated by difference.

Determination of Total Phenolic Compounds (Tpcs): Total crude phenolics were determined using the Folin-Ciocalteau method with slight modification [26].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% Na_2CO_3. After 2 min, 50 µl of 50% Folin-Ciocalteau reagent were 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).

Determination of Tannins: Quantitative estimation of tannins was carried out using the modified vanillin-HCl method [27] as described by [28]. 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.

Determination of Phytate Content: Phytic acid content in legume samples was extracted and estimated according to the procedure described method in [29]. 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.

Statistical Analysis: Data were statistically analyzed using analysis of variance and least significant difference (LSD) using [30]. Significant differences were determined at the 5% level of significance.

Results and Discussion

Chemical Composition

Gross chemical composition of raw studied legumes (faba bean, chick pea, cowpea and soybean) were shown 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. The crude fat ranged from 1.26% in faba bean to 22.33% in soybean. The crude fat content were 1.26, 6.45, 3.16 and 22.33 % on dry weight basis for faba bean, chick pea, cowpea and soybean , respectively. The results showed also that soybean contained the highest level of ash (5.32%) whereas the chickpea has the lowest value (3.07%) compared with other studied legumes. In addition, the results in the same Table indicated that the crude fiber content was higher (6.93%) in faba bean seeds compared with other samples. On the other hand the legumes 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 [31-34].

Table 1: Gross chemical composition of legumes (% on dry weight basis) *

samples	Protien	Fats	Fiber	Ash	Carbohydrate**
Faba bean	29.38	1.26	6.93	3.87	58.56
Chikpea	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 .

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 legumes. Phenolic contents in raw faba bean, chick pea, cowpea and soybean samples were 370.95, 132.59, 763.42 and 249.40 mg GAE/100g, respectively. These results are in agreement with those mentioned by (35-37). Soaking of legumes in distilled water for 12 hours lowered phenolic compounds content compared with control. Soaking processes showed a significant decrease in phenolic content of legumes. 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. [38,39] reported similar results for cowpeas following soaking. Several possible reasons have been suggested for reductions in polyphenol concentrations due to soaking. [40,41] reported that the losses may result simply from leaching into the soak water. Losses may also be attributed to decreases in extractability, as lower molecular weight phenolic compounds polymerize, thus becoming insoluble in water. [42,43] 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 [44,45]. Because of polyphenols are present in the periphery of the seed, there is a possibility of them moving out into the soaking medium through the seed coat [46].

Germination of legumes 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 legumes was decreased gradually during germination period. The reduction level of phenolic compounds in faba bean was, 9.4, 17.8 and 21.4% of its initial values of control after 24, 48 and 72 h of germination, respectively. In chick pea phenolic content decreased after the same periods of germination by 26.9, 37.4 and 43% of its control value, respectively. While, these decrement were about 40.4, 49.5, and 56.9% for cowpea compared with control after the same periods of germination, respectively. And 15.6, 20.1 and 25.2% for soybean of its values in the raw legume after the same germination periods, respectively. These results are in agreement with those mentioned by [38,39]. The reduction in phenolic compounds content during germination may have been a function of the presence of polyphenol oxidase and enzymatic hydrolysis [47,48]. Leaching of poly phenols into the water prior to germination may also partly account for losses.

Tannins

Data in Table (3) shows the effect of soaking and germination on

Table 2. Effect of soaking and germination process on phenolic content in legumes (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.

tannins contents in faba bean, chick pea, cowpea and soybean. Faba bean has higher tannin content than other studied legumes. Tannin contents in faba bean, chick pea, cowpea and soybean raw samples were 684.5,488.1,390.9 and 225.5mg catechin equivalent (CE)/100g, respectively. Similar results were given by [49,50,11].

Soaking

Soaking of legumes in distilled water for 12 hours lowered phenolic content 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, cow pea and soybean, respectively. These results are in the line with those mentioned by [50,11]. The loss in the tannin content after soaking was relatively more due to the fact that those compounds, in addition to their predominance in seed coats [51], are water soluble [52] and consequently leach into the liquid medium. Losses may also be attributed to the presence of water soluble tannins that might have leached in to soaking media, or its hydrolysis by enzymes during soaking period [53,22].

Germination

The results given in Table (3) shown the effect of germination process on tannin content in legumes. A significant reduction was observed in the tannin content of legumes 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. These 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 [49,50,11].

The decrease in the tannin content of legumes following germination may be attributed to the increased activity of poly phenol oxidase and other catabolic enzymes. During germination, activated enzymes result in the hydrolysis of various components. This reduction of tannin content after germination may by attributing to enzymatic hydrolysis [54,41].

Phytic Acid

The effect of soaking and germination on phytic acid content in legumes are shown in Table (4). In raw samples, faba bean has higher phyatic acid content than other investigated legumes. 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 [55,56].

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. The results approved with [21,56,57] The reduction of phytate content in legumes by soaking could be due to the fact that phytic acid in dried legumes exists wholly as a water-soluble salt. This reduction in phytate during soaking of legumes may have been a function of leaching of phytate ions into the soaking water because of the influence of the concentration gradient. Losses may also be attributed to the activation of the endogenous phytase during 12 h of soaking and diffusion of the products. An increase in the phytase activity with a decrease in the level of phytate as a result of soaking in legumes [58]. Germination like soaking germination too resulted in a significant loss of phytic acid in legumes 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 72h 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 37.8,47.7and 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 results are in the same line with those reported by [57]. The reduction of phytic acid in legumes indicated that, an increase in hydrolysis of phytates during germination led to the liberation of inorganic phosphates for plant growth from organic phosphorus containing compound (phytate). The breakdown of phytic acid during germination could be due to increase in the activity of endogenous phytase for its use as source of inorganic phosphate during germination [59-61]. Since phytic acid has been considered to be one of the factors responsible for reducing

Table 3. Effect of soaking and germination process on tannin content in legumes (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
Chicpea	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.

Table 4. Effect of soaking and germination process on phytic acid content in legumes (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
Chicpea	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.

minerals bioavailability, its reduction during germination may have enhanced the nutritional quality of beans.

Conclusions

Soaking (12 h) and germination for (72 h) were required to cause a considerable reduction in the level of phenolic compounds, tannins and phytic acid in the a fore-mentioned studied legumes. Further enhancements in the mentioned parameters were observed up to72 h of germination. This method represent a simple, inexpensive in terms of time, energy and fuel can also be used in household processing of other legumes. In addition, it was essential to improve the nutritional properties of legumes and effectively utilize their full potential as human food.

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