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Influence of Processing and fermentation on chemical composition, total phenolic and phytic acid of some cereals and legumes

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ABSTRACT

The objective of this study was to assess the effect of individual or combined processing treatment methods such as soaking; germination; cooking and fermentation on proximate composition, total phenolic and phytic acid of soybean, chickpea and wheat. Standard methods and analytical procedures were used to analyze proximate composition, total phenolic and phytic acid in raw and after processing treatment methods. The result revealed that, soaking and germination significantly decreased protein, ash and fiber contents, while increased carbohydrate contents. There is no significant change in fat content after processing when compared with raw samples. The processing treatment methods had significant effect on the reduction in total phenolic and phytic acid of soybean; chickpea seeds and wheat grains. In conclusion, individual or combined processing treatment methods can be used for reduction of total phenolic and phytic acid content in soybean, chickpea and wheat thus, enhancing the nutritional value of such beans and grains.

Keywords: Processing, Phytic acid, total phenolic, cereals and legumes.

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INTRODUCTION

Legumes and cereals are widely consumed throughout the world as a source of protein and other nutrients in human diet and animal feed [1]. Supplementation of cereals with high protein legumes, such as chickpea and soybean, is potentially one of the appropriate solutions to protein-calorie malnutrition. Malnutrition is a substantial socioeconomic challenge in healthcare with an estimated prevalence of 30–50%. Malnutrition is associated with increased risk of many complications as well as an increase in direct and indirect costs [2]. Malnutrition is generally used to cover both under-nutrition and over-nutrition. Under-nutrition is one of the most serious problems affecting global health, especially in developing countries [3].

Different techniques of food processing and preparation, such as decortications, soaking, cooking, germination and fermentation, are the major efforts made to reduce the amounts of phytate and tannin content in foods [4,5] and thus improve the nutritive value of legumes and grains. The most effective treatments are fermentation [6] and germination [7]. Germination and fermentation enhance the quality of nutrients, bioactive compounds and decrease the level of anti-nutrients of cereals and legumes [8, 9].

Proteolysis activity of lactic acid bacteria improves availability of proteins and amino acids. Fermentation process contributes to the degradation of phytic acid, which bind divalent cations (zinc, iron, and calcium) and decreasing their bioavailability. Degradation of phytic acid is caused by phytases, which are present in raw material (wheat, rye) and there are produced by lactic acid bacteria also [10,11]. Lactic acid bacteria improve taste and flavor of fermented products via proteolysis and lipolysis activities which cause production of aromatic compounds [12].

The objective of the present research was to investigate the effect of individual or combined processing treatment methods such as soaking; germination; cooking and fermentation on the reduction/elimination of total phenolic and phytic acid content in soybean, chickpea and wheat. This would help in determine simple and cost-effective processing options for developing countries in order to improve the nutritional value of such beans and grains.

MATERIALS AND METHODS

Materials:

Soybean, chickpea seeds and wheat grains will be obtained from the Food Legume Section, Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. Grains were cleaned and other foreign materials were discarded. The cleaned seeds were stored in polyethylene bags under refrigeration until used. All the chemicals used in analysis were of Analytical Reagent grade.

Methods:

Processing methods such as soaking; germination; cooking were done as described by [13] and fermentation as described by Coda *et al.* [14]. Combined processing methods were done in soybean, chickpea and wheat where raw samples were soaked followed by germination; fermentation and cooking.

Soaking: Seeds and grains were soaked in tap water, (seeds : water, 1 : 5 w/v) for 24h at room temperature (30°C). The water left after soaking was discarded. The soaked seeds were washed twice with ordinary water followed by rinsing with distilled water and then dried in a hot air oven at 60°C. Dried samples were ground to (60 mesh sieve) and then stored in air – tight plastic containers for further chemical analysis.

Germination: The seeds and grains were placed in sterile Petri dishes with wet filter paper and kept in an incubator at 30°C for 72h with frequent watering. The sprouted samples were dried in a hot air oven maintained at 60°C, ground (60 mesh sieve) and stored in glass bottles under refrigeration for further analysis.

Cooking: The seeds and grains were cooked in beakers. The ratio of seeds to water was 1: 5 (w/v). The water was allowed to boil before the addition of seeds. The seeds were cooked until soft as felt between fingers. The

cooked samples were then mashed and dried in a hot air oven maintained at 60°C and then ground to a fine powder (60 mesh sieve) and stored.

Natural fermentation: The seeds and grains were soaked in distilled water for 2 days at room temperature. The soaked grains were washed, drained and wet milled (1: 3 w/v) using a laboratory blender, sieved with muslin cloth and fermented for 3 days at room temperature (30°C). The excess water was decanted and a moist paste that was drained to reduce the moisture content obtained. The drained paste was oven dried in hot air at 60°C for 6 to 7 h, after which it was re-milled, sieved and packaged in polyethylene bags put in an airtight container and stored at room temperature prior to analysis.

Moisture, crude protein, crude oil, crude fiber and ash contents will be determined as described by AOAC [15] in raw and processed grains and seeds. The carbohydrate content was determined by difference, that is, addition of moisture, fat crude protein, ash and crude fiber, which was subtracted from 100%. This gave the amount of nitrogen free extract otherwise known as carbohydrate.

%Carbohydrate = 100 - (%Moisture + %Fat + %Ash + %Crude fibre + %Crude protein).

Phytic acid contents of raw and processed samples were determined as described by Wheeler and Ferrel [16]. Phytic acid was extracted from 3g seed flour with 50ml of 3% TCA by shaking at room temperature followed by high speed centrifugation. The phytic acid in supernatant was precipitated as ferric phytate by adding excess ferric chloride and centrifuged. The ferric phytate was converted to ferric hydroxide with a few ml of water and 3ml of 1.5N NaOH, and then the iron content present in the sample was estimated. The phytate phosphorus was calculated from the iron results assuming a 4 : 6 iron : phosphorus molecular ratio. The phytic acid was estimated by multiplying the amount of phytate phosphorus by the factor 3.55 based on the empirical formula C6 P6 O24 H18.

Determination of phenolic content was based on Singleton and Rossi [17] and Song *et al*. [18] while preparation of sample was according to Fu *et al*. [19]. This analysis was based Folin-Ciocalteu assay and using gallic acid as standard. About 1 g of sample was added with 9 ml ethanol: water (50:50, v/v) and was shaken with shaking water bath for an hour followed by filtration by using filter paper (Whatman 41). About 500 µl sample was added into 2500 µl diluted Folin-Ciocalteu (1:10). The mixture was left for 4 minutes and then 2 ml of natrium carbonate (75 g/L) were added. The mixture was incubated at room tempehrature for 2 hours. The absorbance was read at 760 nm. Phenolic content was expressed as gallic acid equivalent (mg GAE/ 100 g) wet weight. Standard concentrations for calibration curve were prepared at 25 to 200 µg/ml.

Statistical analysis

Data were expressed as means ± standard error of the means. The computer SPSS release 16 software was used for calculations (SPSS Inc., Chicago, II, USA). Two-tailed Student's t-test was used to compare the variables at a significance level of $p<0.05$.

RESULTS

The proximate composition (% dry weight) of soybean, chickpea seeds and wheat grains samples during processing are shown in table 1. Moisture content was significantly highest ($p<0.001$) in all processed samples compared to raw samples.

There was a 21.8% reduction in protein content after soaking process of soybean and 12.5% after germination while slightly increased after fermentation (7.3%); cooking (3.5%) and combined processing (9.5%). The results showed 11.6% reduction in protein content after soaking process of chickpea seeds while slightly increased after fermentation (13.4%); cooking (13.0%) and combined processing (11.1%). The results showed significantly lower in protein content after germination process of wheat (18.1%) while protein content increased non-significantly after combined processing (4.7%).

Raw soya bean contained 22.6% fat; raw chickpea seeds contained 3.8% fat and raw wheat contained 3.8% fat where these values reduced non-significantly after different processing as shown in table 1.

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Raw soya bean contained 4.5% crude fiber and it was reduced significantly to 3.5% after fermentation process; 3.6% after cooking process and 3.7% after combined processing. Raw chickpea contained 4.5% crude fiber and it was reduced significantly after different processing methods. Wheat crude fiber content reduced significantly by soaking; fermentation and cooking processing when compared with raw sample.

Ash content was significantly higher ($p<0.05$) in raw samples compared to soaked, germinated, fermented, cooked and combined process samples as showed in Table 1.

Table 1: Proximate composition of soybean, chickpea seeds and wheat grains samples during processing.

Sample	Moisture %	% Dry weight				
		Crude Protein	Crude Fat	Crude Fiber	Ash	NFE
Soybean:						
Raw	11.3±0.3	40.0±0.5	22.6±0.7	4.5±0.1	5.9±0.2	27.0±1.1
Soaked	54.3±2.2 [c]	31.3±0.4 [c]	21.5±0.3	4.6±0.1	5.1±0.1 [b]	37.5±0.8 [c]
Germinated	43.0±1.6 [c]	35.0±0.3 [c]	21.4±0.5	4.2±0.2	4.9±0.1 [c]	34.5±0.7 [c]
Fermented	34.6±1.7 [c]	42.9±0.3 [c]	20.2±0.4	3.5±0.1 [c]	4.4±0.2 [c]	29.0±0.9
Cooked	55.7±1.9 [c]	41.4±0.5	22.1±0.4	3.8±0.1 [c]	4.9±0.1 [c]	27.8±1.2
Combined processing	45.6±2.3 [c]	43.8±0.2 [c]	21.3±0.2	3.7±0.1 [c]	4.9±0.1 [c]	26.3±0.8
Chickpea:						
Raw	11.2±0.4	21.6±0.4	3.8±0.6	4.5±0.2	3.2±0.1	66.9±1.4
Soaked	45.8±1.2 [c]	19.1±0.2 [c]	3.5±0.4	3.7±0.1 [b]	3.0±0.2	70.7±0.6
Germinated	49.4±2.2 [c]	20.5±0.1	3.7±0.2	3.7±0.1 [b]	3.0±0.1	69.1±0.9
Fermented	36.5±2.2 [c]	24.5±0.2 [c]	3.6±0.2	3.6±0.2 [b]	2.8±0.1	65.5±1.2
Cooked	54.2±1.8 [c]	24.4±0.2 [c]	3.5±0.1	3.7±0.1 [b]	3.1±0.1	65.3±0.9
Combined processing	42.3±1.6 [c]	24.0±0.3 [c]	4.0±0.3	3.8±0.1 [b]	3.0±0.1	65.2±0.9
Wheat:						
Raw	11.9±0.3	14.9±0.6	2.9±0.4	3.4±0.1	2.1±0.2	76.7±1.3
Soaked	51.2±2.4 [c]	13.0±0.3	2.6±0.3	2.6±0.1 [a]	2.0±0.1	79.8±1.1
Germinated	41.2±2.2 [c]	12.2±0.2 [c]	2.6±0.2	2.9±0.1	1.7±0.1	80.6±0.9
Fermented	38.5±2.2 [c]	14.9±0.3	2.7±0.2	2.6±0.1 [a]	1.8±0.1	79.0±1.3
Cooked	49.5±1.6 [c]	14.3±0.2	2.5±0.1	2.5±0.1 [a]	1.8±0.1	78.9±0.9
Combined processing	44.2±1.8 [c]	15.6±0.2	2.9±0.2	2.9±0.1	2.0±0.1	75.6±0.9

Values significantly differ from raw sample: [a]: $p < 0.05$, [b]: $p < 0.005$, [c]: $p < 0.001$.

Table 1 showed that carbohydrate content in soaked and germinated was significantly ($p<0.001$) highest compared to raw soya bean but non-significant when compared to fermented, cooked and combined process. Carbohydrate was slightly changed after different process in chickpea and wheat.

Table 2: Total phenolic and Phytate contents in soybean, chickpea seeds and wheat grains samples during processing

Sample	Total Phenolic Content (mg GAE/100g)	Phytate (mg/100g)
Soybean:		
Raw	9.8±0.3	620.0±2.3
Soaked	5.3±0.3 [c]	500.0±2.4 [c]
Germinated	6.8±0.4 [c]	470.0±1.9 [c]
Fermented	6.8±0.4 [c]	460.0±2.5 [c]
Cooked	6.4±0.2 [c]	455.4±1.5 [c]
Combined processing	5.6±0.3 [c]	423.0±1.6 [c]
Chickpea:		
Raw	10.3±0.2	560.0±2.5
Soaked	6.4±0.2 [c]	500.0±1.6 [c]
Germinated	8.2±0.4 [b]	459.0±2.0 [c]
Fermented	8.6±0.2 [c]	448.0±1.8 [c]
Cooked	6.4±0.4 [c]	420.0±2.0 [c]
Combined processing	5.4±0.2 [c]	400.0±2.2 [c]

Wheat:		
Raw	2.9±0.1	590.0±2.5
Soaked	1.8±0.1 [c]	420.0±2.2 [c]
Germinated	2.2±0.1 [b]	412.0±2.2 [c]
Fermented	1.2±0.1 [c]	460.0±3.2 [c]
Cooked	1.6±0.1 [c]	420.0±2.6 [c]
Combined processing	1.6±0.1 [c]	390.0±2.2 [c]

Values significantly differ from raw sample: [a]: $p < 0.05$, [b]: $p < 0.005$, [c]: $p < 0.001$.

As shown in table 2 the processing methods had significant effect on the reduction in total phenolic and phytic acid of soybean; chickpea seeds and wheat grains. Raw chickpea sample showed the highest content of total phenolic (10.3±0.2 mg GAE/ 100g) compared with different raw samples or after different processing methods. Fermented wheat showed the lowest content of total phenolic (1.2±0.1 mg GAE/ 100g). The highest content of phytic acid was observed in raw soybean (620±2.3 g/100g) sample, while combined processing showed the lowest content of phytic acid (390±2.2).

DISCUSSION

The present research evaluates the effect of different processing methods individually or combined on the content of total phenolic and phytic acid in soybean, chickpea and wheat for improving of nutritional value of these legumes and cereals.

In the present study the results revealed that there was a significant reduction in protein content after soaking and germination process of soybean, chickpea seeds while slightly increased after fermentation; cooking and combined processing. The results is in agreement with the results of Van der Riet *et al*. [20] who reported slight increase of protein content after soybean underwent to fermentation process.

In this study, the crude fat of seeds and grains reduced non-significantly when raw samples subjected to the different processing methods. The decrease in fat content after germination may be attributed to their use as an energy source to start germination [21].

The results revealed that crude fiber showed significant reduction in all processing samples compared with raw samples. Reduction in fiber content is usually observed during soaking and fermentation process. These might be due to degradation of fiber into simpler sugars by initiated endogenous enzymes. This observation is in agreement with the previous studies in chickpea, mung bean, kidney bean and quality protein maize based complementary food [21-24].

Ash content was significantly higher in raw samples compared to all processing methods. Similar trend were observed in soaked legumes (*Cejanus cajan, Labla purpureaus and Vignia ungulicalata*) and germinated chickpea [23]. Cooked and soaked soybean showed the same reduction in ash content [25]. Cooking in water reduces the ash seeds and grains due to diffusion of minerals into the cooking water [26]. Processing methods did not significantly altered ash content in chickpea and wheat, while significantly decreased in soybean seed. Wang *et al*. [27] reported a decrease in ash content of peas seed subjected to cooking. These might be due to leaching out of minerals into soaking water. Similar reduction of minerals (Fe, Zn and Ca) were observed in soaked lentil varieties, soaked and germinated *Vigna unguiculata* for poultry feed, germinated chickpea and quality protein maize based complementary food [22, 23, 28].

The carbohydrate content in soaked and germinated was significantly highest compared to raw soybean but non-significant when compared to fermented, cooked and combined process. Carbohydrate was slight differences change after different process in chickpea and wheat. The increase in the Nitrogen free extract might be attributed to loss of soluble solids which increased the concentration of the starch.

The processing methods had significant effect on the reduction in total phenolic and phytic acid of soybean; chickpea and wheat. These might be due to increase in endogenous phytase enzyme activity and leaching of soluble tannin compounds during soaking and was further reduced after germination. Also phenolic content was reduced after soaking process [29]. Phenolic content reduced significantly during tempeh processing (soaking, boiling and fermentation) [30]. Phenolic content was reduced as much as 17% after the

heating process this may be due to the rehydration of the beans as well as the effect of heating and leaching of phenol into boiling water [29, 31]. Soaking process weakens the cell wall tissues of the soybean, which lead to solubilisation of bound polyphenol and release the polyphenol in water [29].

Raw soybean showed the highest phytate content (620 mg/100 g) compared to soaking (500 mg/100 g), boiling (455 mg/100 g) and fermentation (460 mg/100 g). A significant reduction (23-30%) in phytate content was occurred after soaking and boiling [32]. Phytate content was reduced to about 31% after fermentation [33]. The variation in phytate content from different studies may be due to the different cultivars, weather condition and years [34].

CONCLUSIONS

A simple and inexpensive processing technique, improving the nutritional values of soybean, chick bean and wheat by reduces certain anti-nutritional factors as phytic acid and total phenolic. So that it is appropriate technique for production of infant and young children foods.

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