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Influence of Legume Processing Treatments Individually or in Combination on Their Trypsin Inhibitor and Total Phenolic Contents

[1]Rasha Mohamed K, [2]A.Y. Gibriel, [2]Nagwa M. H. Rasmy, [1]Ferial M. Abu-Salem and [1]Esmat A. Abou- Arab

[1]Food Tech. Dept., National Research Centre, Dokki, Cairo, Egypt.
[2]Food Sci. Dept., Fac. of Agric., Ain Shams Univ., Shoubra El-kheima, Cairo, Egypt.

Abstract: Soy bean, mung bean and kidney bean were investigated for their content of anti-nutritional factors (ANFs), including trypsin inhibitors and total phenolic compounds. Longer the periods of soaking caused greater losses in anti-nutritional factors (ANFs) below the control value. The effect of different cooking methods (i.e. boiling, autoclaving, and microwave cooking) on the level of anti-nutritional factors of some legumes were studied. Increasing the period of boiling caused greater losses in trypsin inhibitor activity (TIA). A complete inactivation was achieved in trypsin inhibitor activity for different samples after boiling 90 min and autoclaving at 121°C for10 minute. Autoclaving at 121°C for 10 minute reduced total phenolic compounds by 30.5, 55.8 and 53.4 % over the control value for soybean, mung bean and kidney bean respectively. The maximum reduction in trypsin inhibitor activity (TIA) was obtained after 48- 72 h However, an increase in trypsin inhibitor activity (TIA) was observed in soybean and mung bean as the period of the germination was prolonged for 5 day. The influence of fermentation with four strains of lactic acid bacteria (e.g. Lactobacillus blugaricus, L. casei, L. acidophilus and. L. plantarum on their ant-inutritional factors are studied. Trypsin inhibitor activity (TIA) could not be detected in any of the legume. Results indicated that, as the period of fermentation increased, a significant decrease in total phenolic contents occurred with the different tested strains. The combined treatments were very effective in reducing trypsin inhibitor activity (TIA) and total phenolic compounds.

Key words: Anti-nutritional factors, Legumes, Trypsin inhibitor, Total phenolic compounds.

INTRODUCTION

Legumes play an important role in human nutrition since they are rich source of protein, calories, certain minerals and vitamins. In African diets, legumes are the major contributors of protein and calories for economic and cultural reasons (El Maki *et al*., 2007). However, their role appears to be limited because of several factors including low protein and starch digestibility (Negi *el al.*, 2001), poor mineral bioavailability (Kamchan *et al*., 2004) and high anti-nutrtional factors (ANFs).

The most important ANFs / antiphysiological substances in legumes include protease inhibitors, phenolic substances, non-protein amino acids, lecithins, saponins, flatulence produces and non-starch polysaccharides (Vidivel & Janardhanen, 2001; Olguin *et al*., 2003).

Anti-nutrients have been defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Makkar, 1993).

Protease inhibitors are widespread anti-nutrient substances which block either trypsin or chymotrypsin, thereby reducing digestibility (Adebowale *et al*. 2005). Trypsin inhibitors (TI), which inhibit the proteolytic activity of the digestive enzyme trypsin, can lead to reduced availability of amino acids and reduced growth.

Phenolic compounds, or polyphenols, constitute one of the most numerous and widely distributed groups of substances in the plant kingdom. They can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins. Flavonoids are reported to be the most abundant polyphenols in human diets (Yunfeng *et al.*, 2006). Phenolic compounds or their oxidized products form complexes with essential amino acids, enzymes and other proteins, thus lowering their protein digestibility and nutritional values (Shahidi and Naczk, 1992).

Corresponding Author: Esmat A. Abou- Arab**,** Food Tech. Dept., National Research Centre, Dokki, Cairo, Egypt.
E-mail: eabouarab@yahoo.com

Removals of undesirable components are very essential in improving the nutritional quality and organoleptic acceptability of legumes and in turn help to effectively utilize their potential as human food. Several food processing methods such as germination (Al-Kaisey *et al*., 1997), soaking, dehulling, cooking and fermentation are known to reduce anti-nutritional factors effectively and upgrade the nutritional quality of legumes. The most effective treatments are fermentation and germination (Honke *et al*., 1998) but their applications remain limited because of the additional workload they imply or the particular organoleptic properties they induce.

The aim of this work was to study the effect of some conventional treatments (i.e. soaking, dehulling, and different cooking methods) on reducing anti-nutritional factors (trypsin inhibitors and phenolic compounds) of legumes and evaluation the influence of some biotechnological treatments (i.e. germination and fermentation with lactic acid bacteria for eliminating the anti-nutritional factors in the tested legume. And assess the efficiency of the different processing treatments individually and in combination to suppress the adverse effects of the anti-nutritional components in some legume seeds.

MATERIALS AND METHODS

Materials:

Materials used in this research could be classified as follows:-

Seed Samples:

Soybean (Glycine max. L.), mung bean (Vigna radiata), and kidney bean (Phaseolus vulgaris L.), varieties were obtained from Agriculture Research Center (A.R.C), Ministry of Agriculture, Giza, Egypt. The seeds were thoroughly cleaned from dust and other extraneous materials prior to use.

Bacterial Strains:

Three strains of lactic acid bacteria (LAB), Lactobacillus blugaricus (EMCC1102), L. casei (EMCC1643) and L. acidophilus (EMCC1324) were obtained from the Egyptian Microbial Culture Collection (EMCC) at the Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Lactobacillus plantarum (NRRL B-4004) was obtained from Northern Regional Research Laboratory, USA. These strains were selected according to their abilities to ferment legume seed extracts.

Chemicals:

The chemicals used in this study (gallic acid, tris (hydroxymethyl) aminomethane, trypsin, benzoyl-DL-arginine-p-nitroaniline (BAPA), dimethyl sulfoxide were purchased from Sigma Chemical Company, St. Louis, MO., USA.).

Media:

Lactobacilli Broth:

(MRS-broth) was used to activate as well as to prepare heavy suspension of the investigated bacterial strains. MRS agar was used to activate, maintain and enumerate the cultures of LAB strains according to the method of De Man *et al*., (1960).

Methods:

Processing Treatments:

The processing treatments used for the reduction/elimination of trypsin inhibitor activity (TIA) and total phenolics content were soaking (hydration), dehulling, germination, cooking (i.e., boiling, autoclaving as well as microwave cooking) and fermentation with lactic acid bacteria. After each step for every particular processing treatment, samples were dried at 50°C for 20 h. in a hot air oven and ground in an electric mill to pass from a 60 mesh sieve screen. The powdered samples were stored in plastic containers under refrigeration until analysis for their trypsin inhibitor activity (TIA) and total phenolics content 4°C.

Soaking:

The whole seeds of soybean, mung bean and kidney bean were soaked in distilled water (1:10 w/v) for 12,18 and 24 h at room temperature (25°C).

Dehulling:

Hulls were removed manually after soaking the seeds for different times according to El-Beltagy (1996).

Cooking Methods:

Seeds previously soaked in distilled water for 12 h were drained and rinsed three times with distilled water and then cooked by the methods described below
El-Beltagy (1996).

Boiling:

Seeds were cooked in distilled water (100°C) in the ratio of 1:10 (w/v) on a hot plate for 30, 60 and 90 min.

Autoclaving:

Seeds were autoclaved using (SELECTA) at 15 atmospheric pressure (121°C) in distilled water (1:10 w/v) for 10 min.

Microwave Cooking:

Seeds were placed in a Birex pot with distilled water (1:10 w/v), then cooked in a microwave oven (Sumung 44L-900W) on high for 15 min (about 50% of the seeds were soft when felt between the fingers).

Germination:

Different seeds were sterilized by soaking in ethanol for 1 min., soaked in distilled water (1:10 w/v) for 12 h at room temperature (25 °C), then kept between thick layers of cotton cloth and allowed to germinate in the dark for 1,2,3,4 and 5 days. Germinated seeds were frozen for 12 h to stop the germination process.

Lactic Acid Fermentation:

The milled seed samples were individually mixed with distilled water in a warring blender to obtain slurry ratio of 1:10 (dry legumes: water w/v). 50 ml of each prepared seed slurry were transferred to 100 ml flask and autoclaved for 15 min at 121°C. The investigated LAB strains were activated on MRS agar for 48 h at 37°C. The obtained growth was suspended in 5ml of MRS broth and reinsulated for 24 h at 37°C (activated microbial suspension). The flasks were inoculated with 0.5 ml of activated LAB strains (1 %) and incubated for 72 h at 37°C.

Analytical Methods:

Determination of Trypsin Inhibitor:

Trypsin inhibitor activity (TIA) was measured by the method described by Hamerstrand *et al*., (1981). The trypsin inhibitor content was determined from the following relationship:

$$\text{TI mg /g of sample} = \frac{\text{differential absorbance}}{0.019 \times 1{,}000} \times \text{dilution factor}$$

Determination of Total Phenolics:

Total phenolics were determined from a modified assay described by Chandler and Dodds (1983), which was modified by Shetty *et al*., (1995). The amount of phenolics present in the sample was determined from a standard curve prepared with gallic acid in 95% ethanol .Average values of triplicate estimations were expressed as g/100g sample (dry weight basis).

Statistical Analysis:

Results are expressed as mean value ± standard deviation (S.D) of three replicates. Data were statistically analyzed using analysis of variance and least significant difference using SAS (1985). Significant differences were determined at the 0.05 level of significance.

RESULT AND DISCUSSION

Effect of Physical Treatments on Anti-nutritional Factors of Some Legumes:

Different physical treatments have been proposed to eliminate or reduce anti-nutritional factors in legumes. The physical processing methods including soaking, and cooking effectively improve their nutritive value.

Effect of Soaking on Trypsin Inhibitor:

The result in Table (1) revealed that soaking of soybean, mung bean, and kidney bean and for different periods could lower the level of trypsin inhibitor activity below the control value. Longer period of soaking caused greater losses in trypsin inhibitor activity. On 12 hours soaking, the trypsin inhibitor reached 52.89, 9.60, and 11.41 mg /gm in soybean, mung bean, and kidney bean respectively which corresponding to 9.0, 8.3 and 16.7 % losses. Soaking for 18 h reduced trypsin inhibitor to 50.21, 8.40 and 9.80 mg/g in soybean, mung bean and kidney bean respectively which is corresponding to 13.6, 19.8 and 28.5 % losses. Soaking for 24 hours caused a loss in trypsin inhibitor activity by 15.9, 27.5 and 39.5 % over the control value in soybean, mung bean and kidney bean respectively.

Our results were similar to those obtained by Ramakrishna *et al*., (2006) and Shimelis and Rakshit, (2007) who found that trypsin inhibitors activity was reduced in kidney bean to 9-18 %, by hydration. Khattab and Arntfield (2009) showed that soaking of cowpea, pea and kidney bean seeds significantly reduced their TIA by 10.22-19.85 %. Results also showed variations in the percentage of trypsin inhibitor loss during soaking of different tested seeds and the highest loss was obtained for kidney bean.

Total Phenolic Compounds:

Soaking of different seeds for varying periods had a marked reducing effect on their total phenolic compounds and it was more pronounced with increasing the period of soaking (Table 2).

Soaking for 12 hours caused loss in total phenolic compounds which reached to 19.7, 35.5 and 24.5 % in soybean, mung bean and kidney bean, respectively. The reduction percent of total phenolic compounds in soybean, mung bean and kidney bean reached to 28.0, 46.7 and 33.3 % respectively after soaking for 18 hours.

When the period of soaking was further prolonged to 24 hours a loss in total phenolic compounds reached 31.3, 55.2 and 43.6 % over the control value in soybean, mung bean and kidney bean respectively. These means that soaking treatment was less effective in lowering total phenolic compounds in soybean, than other examined seeds.

Similar tends were reported by Paramjyothi and Anjali (2005) for chickpea, Ramakrishna *et al*., (2006) for mung bean and Khandelwal *et al*., (2010) in Indian pulses. The decrease in free phenolics during soaking may simply be due to leaching out into soaking water along the concentration gradient (Ramakrishna *et al*., 2006).

Xu and Chang (2008 a and b) suggested that the phenomena of the difference of reduction on total phenolic content by soaking might be due to the differences on distribution and content of phenolic compounds in the seed coat and cotyledon between the tested seeds.

Effect of Different Cooking Methods on Trypsin Inhibitor:

Trypsin inhibitor activity (TIA) was significantly ($p<0.05$) decreased by different cooking treatments applied as can be seen in Table (3).

The ordinary boiling of the soaked tested seeds for different time periods brought about a significant decrease in trypsin inhibitory activity as compared with raw seeds. The losses percent of trypsin inhibitory activity were 87.8, 96.8 and 86.5 % in soybean, mung bean and kidney bean, respectively after boiling for 30 min. Increasing the period of boiling caused greater losses in trypsin inhibitor activity. After 60 min. of boiling TIA was drastically decreased (4-10% residual activity) in tested seeds. Complete inactivation of trypsin inhibitor activity was observed for all tested seeds after boiling for 90 min. On the other hand a complete inactivation was achieved in trypsin inhibitor activity for different samples after autoclaving at 121°C for 10 minute.

However a reduction in trypsin inhibitor activity was noticed after microwave cooking for 15 min. for different tested soaked seeds but this loss appeared to be less than that of autoclaving cooking and similar to

Table 1: Effect of soaking on trypsin inhibitor content (mg/ g) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	58.13 [a] ± 0.38	----	10.48[a] ± 0.14	----	13.70[a] ± 0.04	----
			Soaking Periods (hours)			
12	52.89[b] ±0.15	9.0	9.60[b] ± 0.14	8.3	11.41[b] ± 0.13	1.67
18	50.21[c] ± 0.18	13.6	8.40[c] ± 0.28	19.8	9.80[c] ± 0.11	28.5
24	48.89[d] ± 0.17	15.9	7.60[d] ± 0.28	27.5	8.30[d] ± 0.14	39.5

* Means in the same column with different letters are significantly ($p<0.05$) different
** R= Reduction

Table 2: Effect of soaking on total phenol content (mg/100gm) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	40.60[a] ± 0.60	-----	396.31[a] ± 31.71	-----	225.30[a] ± 12.00	-----
			Soaking periods (hours)			
12	32.60[b] ± 0.90	19.7	255.23[b] ± 17.22	35.5	170.10[b] ± 12.00	24.5
18	29.20[c] ± 1.80	28.0	211.44[c] ± 7.70	46.7	150.30[c] ± 2.80	33.3
24	27.90[c] ± 1.20	31.3	177.60[d] ± 6.03	55.2	127.00[d] ± 1.20	43.6

* Means in the same column with different letters are significantly ($p<0.05$) different
** R= Reduction

Table 3: Effect of different cooking methods on trypsin inhibitor content (mg/gm) of soybean, mung bean and kidney bean (dry weight basis)

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	58.13[a] ± 0.38	-----	10.48[a] ± 0.14	-----	13.70[a] ± 0.04	-----
			Boiling periods (mins)			
30	7.10[b] ± 0.14	87.8	0.34[b] ± 0.02	96.8	1.85[b] ± 0.10	86.5
60	2.10[c] ± 0.14	96.4	0.18[c] ± 0.01	98.3	0.52[c] ± 0.02	96.2
90	0.00[d]	100.0	0.00[d]	100.0	0.11[e] ± 0.20	99.2
Autoclaving 10	0.00[d]	100.0	0.00[d]	100.0	0.12[e] ± 0.14	99.2
Microwave 15	1.95[c] ± 0.10	96.6	0.24[bc] ± 0.01	97.7	0.33[d] ± 0.02	97.6

* Means in the same column with different letters are significantly ($p<0.05$) different
** R= Reduction

the loss of trypsin inhibitor activity which happened when the soaked seeds boiled for 6o minute. Similar result were obtained by EL-Adawy (2002) and Alajaji and El-Adawy (2006) who reported that the highest reduction was noted after autoclaving (83.67%) followed by boiling (82.27%), microwave cooking (80.50%) in chickpeas (Cicer arietinum L).

Khattab and Arntfield (2009) stated that boiling, roasting, microwave cooking and autoclaving brought a total removal of trypsin inhibitor of cowpea, pea, and kidney bean. The loss of trypsin inhibitor activity during cooking may be due to destroying by high temperatures, due to their heat-sensitive nature to undetectable amounts when heating processes (cooking and autoclaving) were employed (Shimelis and Rakshit, 2007). The most effective methods for inactivation TIA were boiling (90 min) and autoclaving for 10 min.

Total Phenolic Compounds:

The results of the effect of different cooking methods (i.e. boiling, autoclaving and microwave) on the levels of total phenol content of some legumes are shown in Table (4). The ordinary cooking of the soaked

Table 4: Effect of different cooking methods on total phenolic compounds (mg/100 gm) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	40.60[a] ± 0.60	-----	396.31[a] ± 31.71	-----	225.30[a] ± 12.00	-----
			Boiling priods (mins)			
30	37.80[b] ± 0.62	7.0	239.70[b] ± 6.52	39.5	139.00 [b] ± 0.38	38.3
60	35.90[c] ± 0.50	11.6	217.10[b] ± 12.70	45.2	126.40[c] ± 1.96	44.0
90	30.40[d] ± 1.30	25.51	181.40[cd] ± 3.50	24.2	103.90[d] ± 3.83	54.0
Autoclaving 10	28.20[e] ± 1.60	30.5	175.00[d] ± 15.80	55.8	105.10[d] ± 2.45	53.4
Microwave 15	30.72[d] ± 0.22	24.3	210.20[bc] ± 20.70	4.70	126.50[c] ± 2.54	44.0

* Means in the same column with different letters are significantly ($p<0.05$) different
** R= Reduction

tested seeds for different periods brought about a significant ($p < 0.05$) decrease in total phenolic compounds of all tested seeds as compared with raw seeds. The losses percent of total phenolic compounds were 7.0, 39.5 and 38.3 % in soybean, mung bean and kidney bean, respectively after boiling for 30 min. After 60 min. of boiling total phenolic compounds were drastically decreased in tested seeds by about 11.6, 45.2 and 44.0 % in soybean, mung bean and kidney bean, respectively. Increasing the period of boiling caused more losses in total phenolic compounds.Boiling for 90 minute caused a loss in total phenolic compounds reached to 25.1, 54.2 and 54.0 % over the control value in soybean, mung bean and kidney bean, respectively.

However a slight increase in the reduction of total phenolic compounds was noticed after autoclaving at 121°C for 10 min. than the reduction in total phenolic compounds after boiling for 90 min. which reached to 30.5, 55.8 and 53.4 % over the control value in soybean, mung bean and kidney bean, respectively. On the other hand a reduction in total phenolic compounds was noticed after microwave cooking for 15 min. for tested soaked seeds, but this loss appeared to be less than the autoclaving cooking and it reached to 24.3, 47.0 and 44.0 % for soybean, mung bean and kidney bean, respectively.

Similar results were obtained by Ramakrishna *et al*., (2006), Xu and Chang (2008 a and b) for peas, Kalogeropoulos *et al*., (2010) some dry legumes. Xu and Chang (2008 a and b) reported that the reduction of total phenolic compounds during cooking is not fully understood, however, could be attributed to chemical transformation, decomposition of phenolics, and formation of phenolic-protein complex under thermal and pressure conditions. Also, the soaking and cooking water were discarded, and leaching alone may explain the reductions in total polyphenol (Khandelwal *et al*., 2010). Autoclaving for 10 min. was the most effective method for reducing total phenolic compounds.

Effect of Germination on Trypsin Inhibitor:

The effect of germination process on trypsin inhibitor activity (TIA) of some legumes is given in Table (5). A significant statistical decrease ($p < 0.05$) was observed in the TIA of different tested legumes due to the effect of the germination for 120 h. For the kidney bean, the reduction was 36.5 % in after 24 h. and reached 57.0 % after 72 h. of germination. However, no significant reduction in TIA was observed in kidney bean seeds as the period of germination were prolonged to 120 h.

Likewise, the soybean displayed a reduction of 29.3 % in TIA after 48 h., whereas it was 35.0 % for mung bean in after 72 h. of germination. However, a significant increase in TIA was observed in soybean and mung bean as the period of the germination was prolonged for 120 h.

Raw soybean contained high amount of TIA (58.13 mg/g), which reduced to 46.47 and 41.10 mg/g after 24 and 48 h. of germination, and tended to increase after 72 h. of germination.

Similar trends were observed for mung bean, and the results showed that no significant difference was observed in TIA between raw mung bean and those germinated for 120 h. Oloyo (2004) determined an increase in the TIA in legumes, which was attributed to an increase in the content of phenolic compounds in the germinated seeds.

Table 5: Effect of germination on trypsin inhibitor content (mg/gm) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	58.13[a] ± 0.38	----	10.48[a] ± 0.14	----	13.70[a] ± 0.04	-----
			Germination periods (hours)			
24	46.47[e] ± 0.33	20.0	8.75[b] ± 0.21	16.5	8.70[b] ± 0.14	36.5
48	41.10[f] ± 0.28	29.3	7.30[c] ± 0.14	30.3	7.60[c] ± 0.40	44.5
72	48.85[d] ± 0.21	16.0	6.80[d] ± 0.14	35.0	5.90[d] ± 0.14	57.0
96	53.80[c] ± 0.28	7.4	8.95[b] ± 0.10	14.6	5.80[d] ± 0.14	57.7
120	56.24[b] ± 0.37	3.3	10.15[a] ± 0.21	3.1	5.70[d] ± 0.14	58.4

* Means in the same column with different letters are significantly ($p<0.05$) different
** R= Reduction

The lowest reduction in TIA for the tested germinated legumes was observed for soybean, which reflect the difficultly of TI proteins of soybean to hydrolysis during this process. However, the maximum reduction in TIA was 58.4 % for kidney bean after germination for 120 h.

Similar result was obtained by El-Hag *et al*., (1987) who reported about 50% reduction in trypsin inhibitor activity in kidney bean (P. vulgaris) during 10-day germination.

Ramakrishna *et al*., (2006) found that the raw dry Indian bean had a very high trypsin inhibitory activity which progressively decreased by 51% during the 12 h soaking period which decreased gradually to reach a level of 17% of the basal level of dry seeds at 32h germination. Sangronis and Machado, (2007) reported that the reduction in trypsin inhibitor was 52.5, 25.6 and 41.0 % for white beans, black beans and pigeon beans after germination for 5 days respectively. Germination is a mainly catabolic process as the reserved substances present in the cotyledon are used for the development and growth of the embryo.

However certain studies have shown some contradictory results. Also, Burbano *et al*., (1999) indicate that there is a possibility that the trypsin inhibitors could be utilized as an energy source during the early stages of germination.

Total Phenolic Compounds:

The results in Table (6) showed that germination process causes a reduction percent in total phenolic compounds ranged from 32.8 % after 24 h. of germination to 60.8 % after 120 h. of germination in kidney bean. Longer period of germination caused significant greater losses in total phenolic compounds in kidney bean. For the soybean, the reduction percent in total phenol content was 27.0 % after 24 h. germination and reached 45.0 % after 96 h. of germination. On the other hand, the mung bean displayed high losses of phenolic compounds which reached 66.8% after 48 h.of germination. A decrease in polyphenol contents was observed by Giami *et al*., (2001) for germinated cowpea (41.5 to 51.7%) and for Indian pulses by Khandelwal *et al*., (2010). The reduction of total phenolic compounds during germination may be attributed to the presence of polyphenol-oxidase and enzymatic hydrolysis (Rao and Deosthale, 1982).

However an increase in phenolic compounds was observed in soybean and mung bean during the progressive germination and peaked after 120h. of germination. These means that the highest reduction in phenolic compounds was achieved in after 48, 120 and 72 h. of germination for mung bean 66.8 %, kidney bean 60.8 % and soybean bean 45.0 %, respectively. However, the lowest reduction in phenolic compounds for the tested germinated legumes was observed for soybean after 48 h. of germination.

Also, an increase of total phenols after germination was reported by Khattak *et al*. (2007) for chickpea, Duenas *et al*., (2009) for lupines and Tain *et al*., (2010) for oat.

Lopez-Amoros *et al*., (2006) reported an increase in the antioxidant activity of beans and peas during germination an increase of antioxidant activity after germination was also observed in soybean, adzuki bean and mung bean The changes observed in these legumes were related to the increase of phenolics (Lin and Lai, 2006), and these may be attributed to the biochemical metabolism of seeds during germination.

Duenas *et al*., (2009) reported that germination caused significant changes in the phenolic composition (increasing) due mainly to endogenous enzymes' activation and the complex biochemical metabolism of seeds during this process.

Table 6: Effect of germination on total phenolic compounds (mg/100gm) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	40.60[a] ± 0.60	-----	396.31[a] ± 31.71	------	225.30[a] ± 12.00	------
			Germination periods (hours)			
24	29.70[c] ± 1.50	27.0	131.70[c] ± 10.04	67.0	151.50[b] ± 1.20	32.8
48	24.80[d] ± 0.63	39.0	131.50[c] ± 14.60	66.8	127.70[c] ± 1.99	43.3
72	23.30[e] ± 1.60	45.0	290.60[b] ± 12.10	26.7	113.00[d] ± 2.10	49.8
96	28.50[c] ± 0.84	30.0	291.00[b] ± 32.90	26.6	100.80[e] ± 0.61	55.3
120	35.50[b] ± 1.70	12.6	397.90[a] ± 16.90	0.4+	88.30[f] ± 6.50	60.8

* Means in the same column with different letters are significantly ($p<0.05$) different
** R= Reduction

Effect of Fermentation with Lactic Acid Bacteria:

The influence of fermentation of soybean, mung bean and kidney bean legumes acid bacteria (e.g. Lactobacillus blugaricus (EMCC1102), L. casei (EMCC1643), L. acidophilus (EMCC1324) and. L. plantarum (NRRL B-4004)) on their anti-nutritional factors are studied.

Trypsin Inhibitor:

The effect of fermentation with four strains of lactic acid bacteria for 72 h at 37ºC on trypsin inhibitor activity of autoclaved legumes (at 1:10 dry seed: water) are studied. Before fermentation, different seeds slurry was subjected to moist sterilization and this process completely destroyed trypsin inhibitor as can be seen in Table (3).Therefore, trypsin inhibitor activity could not be detected in any of the legumes fermented with one culture of lactic acid bacteria.

Trypsin inhibitor is known to be heat labile. Complete destruction of trypsin inhibitor activity after autoclaving has been reported in yam and lima beans (Agunbiada and longe, 1996; Apata and Ologhobo, 1997). Moist heating generally destroys the trypsin inhibitor.

Total Phenolic Compounds:

The effect of fermentation of legumes (1 legume: 10 water) with L.plantarum, L.bulgaricus, L.acidophilus and L.casei at 37°C for 72 on their total phenolic contents are given in Tables (7, 8, 9, 10). The results indicated that, longer period of fermentation caused greater losses in total phenolic content occurred with the different tested strains. A significant ($p < 0.05$) reduce in total phenolic content was first observed at 24 h fermentation with L. plantarum (26.6, 69.1 and 44.5 %) for soybean ,mung bean and kidney bean legumes respectively . However, further significant reductions in total phenolic contents were obtained with prolonged of fermentation process. The reduction reached 67.0, 77.4 and 74.6 % after 72 h of fermentation with L. plantarum for soybean, mung bean, and kidney bean legumes respectively (Table 7). The same pattern was found for the other tested strains (Tables 8, 9 and 10). Total phenolic content reduced from 40.60 to 19.80, 10.10 and 15.30 mg/100 gm (51.2, 75.4 and 62.3 % reduction) for soybean fermented for 72 h with L. bulgaricus, L. acidophilus and L. casei respectively.

However, fermentation of mung bean and kidney bean with L .bulgaricus for 72 h significantly reduced phenolic content by 75.0 and 71.6 % respectively and the reduction was 75.2 and 79.6 % for the same samples when fermented with L. acidophilus. Fermentation of soybean, mung bean and kidney bean legumes with L. acidophilus at 37 ºC for 72 h significantly reduced total phenolic by 75.4, 75.2 and 79.6 % respectively.

Mahgoub and Elhag (1998) and Rani and Khetarpaul (1999) has been reported a reduction in total phenolic content during fermentation of some legumes and attributed this reduction to the activity of polyphenol oxidase present in the food grain or microflora.

Rodríguez *et al*. (2009) reported that degradation of phenolic compounds in LAB has not been completely described. Results showed that lactic acid bacteria was able to degrade some food phenolic compounds giving compounds influencing food aroma as well as compounds presenting increased antioxidant activity.

Table 7: Effect of fermentation with L.plantarum on total phenol content (mg/100gm) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	40.60[a] ± 0.60	----	396.30[a] ± 31.71	----	225.30[a] ± 12.00	----
			Fermentation periods (hours)			
24	29.80[b] ± 0.86	26.6	122.40[b] ± 18.20	69.1	125.10[b] ± 4.10	44.5
48	20.00[c] ± 0.80	50.7	97.20[b] ± 14.8	75.5	92.10[c] ± 0.24	59.1
72	13.40[d] ± 1.10	67.0	89.60[b] ± 7.44	77.4	57.30[d] ± 1.02	74.6

* Means in the same column with different letters are significantly ($p<0.05$) different
** R= Reduction

Table 8: Effect of fermentation with L.bulgaricus on total phenol content (mg/100gm) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	40.60[a] ± 0.60	----	396.30[a] ± 31.71	----	225.30[a] ± 12.00	----
			Fermentation periods (hours)			
24	33.60[b] ± 0.62	17.2	336.30[b] ± 36.40	15.1	133.69[b] ± 1.30	40.7
48	28.30[c] ± 0.40	30.3	179.43[c] ± 8.42	54.7	81.90[c] ± 12.73	63.7
72	19.80[d] ± 0.89	51.2	99.53[d] ± 10.20	75.0	64.00[d] ± 2.90	71.6

* Means in the same column with different letters are significantly ($p<0.05$) different
** R= Reduction

Table 9: Effect of fermentation with L.acidophilus on total phenol content (mg/100gm) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	40.60[a] ± 0.60	----	396.30[a] ± 31.71	----	225.30[a] ± 12.00	----
			Fermentation periods (hours)			
24	26.62[b] ± 0.82	34.4	280.03[b] ± 14.70	29.3	121.72[b] ± 7.50	46.0
48	16.20[c] ± 1.71	60.1	138.63[c] ± 23.50	65.0	75.10[c] ± 3.90	66.7
72	10.10[d] ± 0.40	75.4	98.46[c] ± 20.22	75.2	45.96[d] ± 0.78	79.6

* Means in the same column with different letters are significantly ($p<0.05$) different
** R= Reduction

Table 10: Effect of fermentation with L. casei on total phenol content (mg/100gm) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	40.60[a] ± 0.60	----	396.30[a] ± 31.71	----	225.30[a] ± 12.00	----
			Fermentation periods (hours)			
24	30.10[b] ± 0.44	26.0	312.63[b] ± 10.80	21.1	134.20[b] ± 0.63	40.4
48	21.90[c] ± 2.7	46.1	175.33[c] ± 62.30	55.8	80.33[c] ± 1.70	64.4
72	15.30[d] ± 0.46	62.3	97.30[d] ± 4.10	75.5	61.93[d] ± 1.90	72.5

* Means in the same column with different letters are significantly ($p<0.05$) differen
** R= Reduction

Effect of Some Combined Treatments on Anti-nutritional Factors of Soybean, Mung Bean and Kidney Bean Legumes:

Most researchers have studied the effect of individual processing methods on nutritional quality and anti-nutritional factors of legumes but information on the effect of combined processes on improvement of nutritional quality of legumes is scarce. Therefore, the effect of combined treatments in comparison with the most effective individual one for reducing the anti-nutritional factors of some legumes was studied.

Trypsin Inhibitor Activity:

The combined treatment of soaking and dehulling significantly ($p<0.05$) affected the trypsin inhibitor activity of the tested seeds as can be seen in Table (11). Dehulling after soaking 24 hours caused loss in TIA reached by 22.3, 42.3 and 47.4 % in soybean, mung bean and kidney bean legumes, respectively. Similar results were obtained by Mubarak (2005) for mung bean seeds and Wang *et al*., (2009) for lentils.

Similar results were obtained by Mubarak (2005) who found that Trypsin inhibitor activity was significantly ($p < 0:05$) decreased by 15.8 % with soaking and dehulling of mung bean seeds. The results in Table (11) showed that trypsin inhibitor, due to their heat-sensitive nature was significantly reduced to undetectable amounts by the heating process (boiling and autoclaving).

Our results are accordance with those obtained by EL-Adawy 2002; Mubarak (2005) and Jourdan *et al*., (2007). Dehulling of germinated seeds contributed significantly ($P> 0.05$) towards lowering down trypsin inhibitor by 41.0, 22.3 and 69.0 % for soybean, mung bean and kidney bean respectively. On boiling of germinated seeds for 30 min, complete inactivation was observed for all tested samples. The same trend was observed when legumes subjected to autoclaving before fermentation with lactic acid bacteria. Ramakrishna *et al*., (2006) reported that the trypsin inhibitor activity of raw legume seeds was found to be decreased by all cooking methods employed.

Total Phenolic Compounds:

It has been known that soaking, cooking, germination and fermentation improve the quality of legumes because of the removal of some anti-nutritional factors. In many instances, usage of only one method may not affect of desired removal of anti-nutritional factors and a combination of two or more methods is required. Therefore, the effect of different combined treatments for reducing the total phenol content of some legume are studied. The result in Table (12) showed that dehulling of soaked seeds significantly lowered the total phenolics but the loss appeared to be less in soybean that the other tested seeds. The reduction of total phenolic content ranged from 42.6 to 62.4 for dehulled -soaked soybean and kidney bean. These results are agree with those of Deshpande and Cheryan (1983) and Kataria *et al*., (1989) reported that soaking followed by dehulling had the most pronounced effect on the reduction of polyphenols.

Table 11: Effect of combined processing methods on trypsin inhibitor (mg/g) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	58.13^{Aa} ± 0.38	----	10.48^{Ca} ± 0.14	----	13.70^{Ba} ± 0.04	----
Soaking (24 h)	48.89^{Ab} ± 0.17	15.9	7.60^{Cb} ± 0.28	27.5	8.30^{Bb} ± 0.14	39.4
Soaking 24h + Dehulling	45.15^{Ac} ± 0.21	22.3	6.05^{Cd} ± 0.21	42.3	7.20^{Bc} ± 0.14	47.4
			Cooking			
Boiling (90 min)	0.00^{Bf}	100.0	0.00^{Bf}	100.0	0.11^{Af}	99.2
			Autoclaving			
(10 min)	0.00^{Bf}	100.0	0.00^{Bf}	100.0	0.12^{Af} ± 0.14	99.1
			Germination			
	41.10^{Ad} ± 0.28 48h.	29.3	6.80^{Bc} ± 0.14 72h.	35.1	5.70^{Cd} ± 0.14 120h.	58.4
Germination + dehulling	34.30^{Ae} ± 0.10	41.0	5.60^{Be} ± 0.004	22.3	4.25^{Ce} ± 0.21	69.0
Germination+ boiling (30 min)	0.00^{Af}	100.0	0.00^{Af}	100.0	0.00^{Af}	100.0
Germination+ehulling + boiling (30 min)	0.00^{Af}	100.0	0.00^{Af}	100.0	0.00^{Af}	100.0
Autoclaving + Fermentation by lactic acid bacteria (LAB)	0.00^{Af}	100.0	0.00^{Af}	100.0	0.00^{Af}	100.0

* Means in the same column with different small letters are significantly ($p<0.05$) different

* Means in the same row with capital different letters are significantly ($p<0.05$) different

** R = Reduction

As mentioned before a significant ($p< 0.05$) decrease in total phenolic compounds was first observed after autoclaving followed by fermentation with L. acidophilus at 37°C for 72 h appeared to more beneficial for lowering down the total phenolic compound of autoclaved soy, mung and kidney bean were 30.5, 55.8, 53.4

mg/ 100 g sample respectively, which reduced to 75.4, 75.2 and 79.6 mg/ 100 g after fermentation with L. acidophilus for the same samples (75.4-79.6 % losses). These results are accordance with those obtained by Towo *et al*., 2006) who reported that phenolic compounds of legumes and grains were significantly reduced by fermentation.

Lactic acid fermentation has been shown to decrease the total phenolic content (Hassan and El Tinay, 1995). The decrease in phenolic compounds during fermentation could be due to the polyphenol oxidase activity either from the seeds or microflora and to the acidic environment that may result in abstraction of hydride ions and rearrangement of the phenolic structures (Chen *et al*., 2001).

Table 12: Effect of different processing methods on total phenolic content (mg/100g) of soybean, mung bean and kidney bean (dry weight basis).

Treatments	Soybean	R %	Mung bean	R %	Kidney bean	R %
Raw seeds	40.6^{Ca} ± 0.60	----	396.31^{Aa} ± 31.71	----	2225.3^{Ba} ± 12	----
Soaking (24 h)	27.90^{Cc} ± 1.2	31.3	177.60^{Ab} ± 6.03	55.2	127.00^{Bb} ± 1.2	43.6

Table 12: Continue.

Soaking 24h + Dehulling	22.3^{Cd} ± 1.5	42.6	105.40^{Ab} ± 7.93	73.4	84.8^{Bd} ± 1.9	
Cooking	30.4^{Cb}	25.1	181.40^{Ab}	54.2	103.9^{Bc}	54.0
Boiling (90 min)	± 1.3		± 3.5		± 3.83	
Autoclaving	28.2^{Cc}	30.5	175.0^{Ab}	55.8	105.1^{Bc}	53.4
At 121 °C /10 min	± 1.6		± 15.8		± 2.45	
Germination	22.3^{Cd} ± 1.6 72h.	45.1	131.50^{Ac} ± 14.6 78h.	66.8	88.3^{Bd} ± 6.50 120h.	60.8
Germination + dehulling	19.0^{Be} ± 0.85	53.2	80.1^{Ad} ± 32.9	79.8	64.3^{Ae} ± 0.77	71.5
Germination+ boiling (30 min)	19.2^{Be} ± 0.61	5.27	96.02^{Ad} ± 14.4	75.8	81.73 ± 1.8	63.7
Germination+ehulling + boiling (30 min)	16.5^{Cf} ± 0.40	59.4	75.5^{Ad} ± 6.28	81.0	57.2^{Be} ± 3.85	74.6
Autoclaving + Fermentation by Lactobacillus acidophilus	10.1^{Cg} ± 0.4	75.4	98.46^{Ad} ± 20.22	75.2	45.96^{Bf} ± 0.78	79.6

* Means in the same column with different small letters are significantly ($p<0.05$) different
* Means in the same row with capital different letters are significantly ($p<0.05$) different
** R = Reduction

Conclusion:

It could be concluded that longer the periods of soaking caused greater losses in anti-nutritional factors (ANFs) below the control value. Increasing the period of boiling caused greater losses in trypsin inhibitor activity (TIA). A complete inactivation was achieved in trypsin inhibitor activity for different samples after boiling 90 min and autoclaving at 121°C for10 minute. The period of fermentation increased, a significant decrease in total phenolic contents occurred with the different tested strains. The combined treatments were very effective in reducing TIA and total phenolic compounds.

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