

## Effect of Heat Treatments on Certain Antinutrients and *in vitro* Protein Digestibility of Peanut and Sesame Seeds

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**The effect of heat treatments (boiling, autoclaving, microwave cooking and roasting) on the levels of certain antinutritional factors (phytic acid, trypsin inhibitor,  $\alpha$ -amylase inhibitor, lectin activity and tannins) and *in vitro* protein digestibility (IVPD) of peanut and sesame seeds were investigated. All heat treatments significantly reduced the levels of all the investigated antinutrients and improved the IVPD of peanut seeds. Of the attempted treatments, autoclaving, boiling, roasting-salting and oil-roasting were the most effective in reducing the levels of antinutrients and improving IVPD of peanut. Roasting in both brown and white sesame seeds partially eliminated the studied antinutrients (the reduction ranged from 15.6% to 61.2% in all antinutrients) and improved IVPD (increased by 10% and 9.1%, respectively). Also, Tehineh (sesame butter-like) contained lower levels of antinutrients than raw sesame seeds and exhibited a higher IVPD (82.8%).**

Keywords: peanut, sesame, antinutritional factors, autoclaving, microwave, boiling, roasting

### Introduction

Peanut and sesame are two of world's most important oil seed crops and they offer good sources of edible oil and nutritious food for humans. In Egypt peanut and sesame are consumed mostly as processed products and there are many popular foods prepared from them. Sesame is used to make "Dokka" (a popular food in Egypt prepared domestically from roasted sesame) and sesame butter-like that is called "Tehineh" (a local food in the Middle East). Tehineh is the product of the milled seeds of sesame, which were dehulled and roasted without adding or removing any of its constituents. Tehineh is used in preparation of some local dishes such as java beans, chick peas (hommus tehineh), salads and in desserts such as honey and palm-date molasses (Abu-Jdayil *et al.*, 2002). Also sesame is used in bakeries, confectionaries and in the formulation of baby food. Peanut is consumed as boiled, roasted, fried, used to make peanut butter and used (crude or roasted) in other products like confectioneries.

Peanut and sesame seeds are good sources of proteins, complex carbohydrates and some minerals, additionally; they are good sources of energy due to their high contents

of fats (Ejigui *et al.*, 2005; Kanu *et al.*, 2007). Also, peanut and sesame seeds contain certain bioactive compounds (like isoflavones and sesamin) that may also play a role in the reduction of the risk for the development of chronic diseases such as cancer, diabetes, and coronary heart diseases (Chukwumah *et al.*, 2007; Kanu *et al.*, 2007). However, their nutritional quality is limited by the presence of antinutritional factors that exhibit undesirable physiological effects (Ejigui *et al.*, 2005; Kanu *et al.*, 2007). The antinutritional factors (ANFs), being structurally different compounds are broadly divided into two categories: proteins (such as lectins and protease inhibitors) and others such as phytic acid, tannins, oligosaccharides, saponins and alkaloids (Martín-Cabrejas *et al.*, 2009). Antinutritional factors may also be classified according to their ability to withstand thermal processing, the most commonly employed treatment for destroying them. Heat labile factors include some components like protease inhibitors, lectins, and goitrogens, whereas heat stable factors are represented by saponins, non-starch polysaccharides, antigenic proteins, estrogens and some phenolic compounds (Francis *et al.*, 2001). The negative impacts of the ingestion of antinutritional factors have extensively been reported. For instance, some factors, like trypsin inhibitor, affect protein utilization and digestion, others, like phytic acid and tannins,

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affect mineral utilization. Also, lectin causes disruption of the small intestinal metabolism and morphological damage to the villi (Grant, 1991; Francis *et al.*, 2001).

Processing normally affects factors, such as trypsin inhibitors and phytic acid contents, which in turn can enhance or reduce the bioavailability of proteins and minerals (Nestares *et al.*, 1999). Some antinutritional factors may exert beneficial health effects at low concentrations. Thus, the manipulation of processing conditions and removal or reduction of certain unwanted components of food may be required (Shahidi, 1997). The most common domestic processing methods include ordinary and pressure cooking. Microwave heating is increasing and its use for cooking is becoming popular, due to the reduction of processing time (Habiba, 2002). The levels of trypsin inhibitor activity,  $\alpha$ -amylase inhibitor activity, phytic acid, tannins and lectins were found to be decreased to a considerable extent during heat processing especially moist heat (cooking, extrusion, autoclaving and microwave) (Habiba, 2002; Wang *et al.*, 2008; Embaby, 2010). Also, considerable decreases were observed in phytic acid, trypsin inhibitor activity and tannins after roasting (Fagbemi *et al.*, 2005; Frontela *et al.*, 2008).

The information on heat processing effects on the antinutritional factors in peanut and sesame seeds is scarce. With this prospect, an attempt has been made to find out the effects of heat processing (boiling, autoclaving, microwave, roasting) on certain antinutritional factors (trypsin inhibitor,  $\alpha$ -amylase inhibitor, phytic acid, lectin and tannins) and *in vitro* protein digestibility in peanut and sesame seeds.

## Materials and Methods

**Materials** Peanut seeds (*Arachis hypogaea*) and two kinds of sesame seeds (*Sesamum indicum* L., white and brown seeds) were obtained from agronomy department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Tehineh samples (produced by Rashidi Company, Egypt) were purchased from a local market at Ismailia City. Trypsin (from bovine pancreas),  $\alpha$ -amylase (from porcine pancreas), *N* $\alpha$ -benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPNA), Neuraminidase and phytic acid were from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade.

### Sample preparation and processing methods

**Boiling** In-shell peanuts were boiled (100°C) in the peanut:water ratio of 1:5 (w/v) for 20 and 40 min.

**Autoclaving** In-shell peanuts were autoclaved (121°C) in the peanut:water ratio of 1:5 (w/v) for 10 and 20 min.

**Microwave** In-shell peanuts were microwave cooked at 2450 MHz. in the peanut:water ratio of 1:5 (w/v) for 6 and

12 min using a domestic size Moulinex microwave Model Microchef 2335 type 907. After boiling, autoclaving and microwave treatments the peanuts were allowed to cool to room temperature and then shelled (without remove the skin from the kernels). The kernels were dried at 50°C for 12 h and the dried seeds were ground into flour to pass a 0.5 mm sieve.

**Roasting** Samples were divided into five groups.

**Roasting in-shell peanuts** Five hundred grams of In-shell peanuts were roasted in a preheated oven at  $180 \pm 5^\circ\text{C}$  for 20 min with periodic shaking for even roasting.

**Roasting shelled peanuts** Five hundred grams of peanut kernels were roasted in a preheated oven at  $180 \pm 5^\circ\text{C}$  for 20 min with periodic shaking for even roasting.

**Roasting-salting the shelled peanuts** Five hundred grams of peanut kernels were roasted in a preheated oven at  $180 \pm 5^\circ\text{C}$  for 15 min then a small amount of 5% salt water (50 mL salt water/kg kernels) was sprinkled over peanuts and again roasted for 5 min to get rid of the excess moisture.

**Oil-roasting** Five hundred grams of peanuts kernels were deep fried in preheated frying oil at  $140 \pm 5^\circ\text{C}$  for 10 min with periodic agitation for even roasting.

**Roasting Sesame** Five hundred grams of white or brown sesame seeds were roasted (using a roasting pan) for 10 min (until lightly browned). All roasted samples (peanut and sesame seeds) were allowed to cool to room temperature, and then the roasted In-shell peanuts were shelled. The samples were ground into flour to pass a 0.5 mm sieve.

### Analyses

**Analysis of phytic acid** Phytic acid was determined according to the method described by Latta and Eskin (1980), and later modified by Vaintraub and Lapteva (1988). One gram of dried sample was extracted with 50 mL 2.4% HCl for 1 h at ambient temperature and centrifuged ( $3000 \times g$  / 30 min). The clear supernatant was used for the phytate estimation. One milliliter of Wade reagent (0.03% solution of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  containing 0.3% sulfosalicylic acid in water) was added to 3 mL of the sample solution and the mixture was centrifuged. The absorbance at 500 nm was measured using a spectrophotometer (6505 UV/Vis, Jenway LTD., U.K.). The phytate concentration was calculated from the difference between the absorbance of the control (3 mL of water + 1 mL Wade reagent) and that of assayed sample. The concentration of phytate was calculated using phytic acid standard curve and results were expressed as g phytic acid per 100 g dry matter. To prepare the phytic acid standard curve, a series of standard solutions were prepared containing 5 – 40 mg/mL phytic acid in water (Latta and Eskin, 1980). Three milliliters of the standards were pipetted into 15 mL centrifuge tubes with 3 mL of water used as a zero

level. To each tube was added 1 mL of the wade reagent, and the solution was mixed on a vortex mixer for 5 s. The mixture was centrifuged for 10 min and the supernatant read at 500 nm (with no incubation time) by using water to zero the spectrophotometer.

**Analysis of  $\alpha$ -amylase inhibitor**  $\alpha$ -Amylase inhibitor activity (AIA) was evaluated according to the method of Alonso *et al.* (1998). One gram sample was extracted with 10 mL of deionized water for 12 h at 4°C and the supernatant was tested for AIA: 0.25 mL of sample solution containing the inhibitor was incubated with 0.25 mL of  $\alpha$ -amylase enzyme solution (0.003% in 0.2 M sodium phosphate buffer, pH 7.0, and containing 0.006 M NaCl) for 15 min at 37°C. To this mixture was added 0.5 mL of 1% starch solution (gelatinized) which was pre incubated at 37°C. At the end of 3 min, the reaction was stopped by the addition of 2 mL of dinitrosalicylic acid reagent and heating in a boiling water bath for 10 min. The absorbance was recorded at 540 nm. One unit of enzyme activity was defined as that which liberates, from soluble starch, one micromole of reducing groups per min at 37°C and pH 7.0 under the specified conditions. However, no  $\alpha$ -amylase inhibitor activity against pancreatic amylase was detected in the tested samples.

**Analysis of tannins** Tannins contents of the seed flours were determined by the procedure of AOAC (1990). Two hundred milligrams of the sample was extracted with 10 mL of 70% aqueous acetone (v/v) for 24 h at room temperature. The extracts were centrifuged at 3000  $\times$  g for 20 min and the supernatant was analyzed tannins. In a test tube 0.5 mL of the tannins extract and 1 mL of saturated sodium carbonate solution were added to 0.5 mL Folin- Denis reagent. The volume was made up to 10 mL with distilled water. After 30 min the tannins content was measured at 760 nm with the spectrophotometer against experimental blank adjusted to zero absorbance. Tannic acid was used as a standard compound.

**Analysis of trypsin inhibitor** Trypsin inhibitor activities were determined using the procedure of Kakade *et al.* (1974). One gram of defatted seed flour was mixed with 100 mL of 0.009 M HCl. The mixture was shaken at ambient temperature for 2 h and centrifuged (10000  $\times$  g, 20 min) and the supernatant was used for inhibitor estimation. The extract from each sample was diluted with distilled water to obtain a dilution whereby 1 mL extract produced trypsin inhibition activity of between 40-60%, such dilution was used. The extract (1 mL) was incubated with 1 mL of trypsin solution at 37°C for 10 min. A 2.5 mL of prewarmed substrate (BAPNA) was added and after exactly 10 min at 37°C the reaction was stopped with 0.5 mL of acetic acid (30%, v/v). The absorbance was measured at 410 nm against a blank using the spectrophotometer.

The inhibitor content was calculated from the deferential absorbance readings and reported in pure or absolute units as milligram of trypsin inhibitor per gram of sample by using the following equation derived by Hamerstand *et al.* (1981)

$$\text{TI, mg/g of sample} = \frac{A_{\text{std}} - A_{\text{sam}}}{0.019 \times \text{sample wt., g}} \times \frac{\text{dilution factor}}{1,000 \times \text{sample size, mL}}$$

**Lectin activity** One gram of defatted seed flours was mixed with 25 mL of pre-cooled 1% acetic acid (for sesame seeds) or with phosphate-buffered saline (PBS), pH 7.2 (for peanut seeds) in an Ultra-turrax (T50) homogenizer at maximum speed for 5 min at room temperature. The homogenate was centrifuged at 2000  $\times$  g for 30 min at 4°C. The supernatant contained the raw extracts of lectin from sesame and peanut. Lectin activity from sesame seeds was assayed using erythrocytes of albino rat which were washed three times with PBS and used as a 3% (v/v) suspension in the same buffer. A 3% suspension (in PBS, v/v) of washed human erythrocytes, types A, B and O were pretreated with neuraminidase (0.2 units of Sigma Co. N-2876 neuraminidase enzyme per 10 mL blood cell suspension for 1 h at 37°C) then with trypsin (1000 units per 10 mL blood cell suspension for 1 h at 37°C) to assay the lectin activity from peanut seeds. Agglutination was determined using the procedure of Kortt (1984). Starting with 50  $\mu$ L aliquots of the extracts, serial two-fold dilution were made with PBS in styrene u-well microtiter trays. A 3% solution of erythrocytes was added to each well for a final volume of 100  $\mu$ L and the hemagglutination titer was recorded after 1 h at room temperature as the reciprocal of the highest dilution giving visible agglutination.

**In vitro protein digestibility (IVPD)** The IVPD of both the raw and processed seed samples was measured according to the multi-enzyme technique (Hsu *et al.*, 1977) and calculated by using the following equation:

$$Y = 210.464 - 18.1x$$

where  $Y$  is the percentage of protein digestibility and  $x$  is the pH of the protein suspension after 10 min digestion with a three enzyme solution.

**Statistical analysis** The results are given as means plus or minus SD and they were subjected to a one-way analysis of variance (ANOVA) using SPSS 13 computer program (IBM Co., Chicago, IL, USA). Means comparisons were performed using Duncan's Multiple Range Test and differences were considered significant at  $p < 0.05$ .

## Results and Discussion

**Effect of heat treatments on phytic acid** The phytate molecule is negatively charged at the physiological pH and

is reported to bind essential, nutritionally important divalent cations, such as iron, zinc, magnesium and calcium. This forms insoluble complexes, thereby making minerals unavailable for absorption (Frontela *et al.*, 2008). Data on phytic acid contents of raw and processed peanut seeds are summarized in tables 1 and 2. The level of phytic acid in raw peanut seeds was 2.63% and a significant reduction ( $p < 0.05$ ) was observed after all heat treatments (boiling, microwave, autoclaving, roasting). In general, longer time of boiling, microwave and autoclaving resulted in lower levels of phytic acid. Thus, autoclaving for 20 min was the most effective for phytic acid reduction (24.7% loss) (Table 1). Roasting treatments were more effective (the reduction ranged from 15.6%

to 22%) than boiling and microwave (the reduction ranged from 3.8% to 11.8%) for phytic acid reduction (Table 2). Raw brown sesame seeds contained a higher level of phytic acid (6.5%) than that reported by Lott *et al.* (2000) (4.71%) and Reddy (2002) (5.36%), but raw white sesame seeds contained a lower level (4.2%) (Table 3). Roasting caused a significant reduction in phytic acid contents in both brown and white sesame seeds (the reductions were 23.1 and 28.6 %, respectively). Also, Tehineh (sesame seeds were dehulled and roasted during the preparation) contained a high level of phytic acid (3.7%) (Table 3). Similarly, a significant reduction of phytic acid contents by thermal processing (roasting, cooking, autoclaving and microwave) has been observed in

**Table 1.** Effects of boiling, microwave and autoclaving on phytic acid (g/100 g), tannins (mg/g) and trypsin inhibitor activity (TIA, mg/g) in peanut seeds\*.

Treatment	Phytic acid	Loss (%)	Tannins	Loss (%)	TIA	Loss (%)
<b>Raw seed</b>	2.63 ± 0.06 a		8.9 ± 0.10 a		5.6 ± 0.26 a	
<b>Boiling</b>						
20 min	2.53 ± 0.06 ab	3.8	7.2 ± 0.16 b	19.1	1.5 ± 0.37 b	73.2
40 min	2.39 ± 0.06 c	9.1	5.2 ± 0.26 c	41.6	0.00 ± 0.00 c	100
<b>Microwave</b>						
6 min	2.52 ± 0.10 b	4.2	8.3 ± 0.31 d	6.7	4.4 ± 0.16 d	21.4
12 min	2.32 ± 0.04 c	11.8	7.3 ± 0.16 b	18.0	2.1 ± 0.12 e	61.5
<b>Autoclaving</b>						
10 min	2.38 ± 0.03 c	9.5	3.8 ± 0.16 e	57.3	0.41 ± 0.08 f	92.7
20 min	1.98 ± 0.06 d	24.7	2.8 ± 0.27 f	68.5	0.00 ± 0.00 c	100

\* Mean values of each column followed by different letter are significantly different at  $p < 0.05$ .

**Table 2.** Effects of roasting on phytic acid (g/100 g), tannins (mg/g) and trypsin inhibitor activity (TIA, mg/g) in peanut seeds\*.

Treatment	Phytic acid	Loss (%)	Tannins	Loss (%)	TIA	Loss (%)
Raw	2.63 ± 0.06 a		8.9 ± 0.10 a		5.6 ± 0.26 a	
Roasting-shelled	2.22 ± 0.06 b	15.6	7.3 ± 0.04 b	18.0	3.5 ± 0.11 b	37.5
Roasting-salting	2.05 ± 0.09 c	22.0	6.0 ± 0.19 c	32.6	0.54 ± 0.24 c	90.4
Roasting in-shell	2.19 ± 0.05 bc	16.7	7.5 ± 0.11 b	15.7	3.8 ± 0.13 b	32.1
Oil-roasting	2.13 ± 0.15 bc	19.0	5.7 ± 0.24 c	36.0	0.64 ± 0.081 c	88.6

\* Mean values of each column followed by different letter are significantly different at  $p < 0.05$ .

**Table 3.** Effect of roasting on phytic acid (g/100 g), tannins (mg/g) and trypsin inhibitor activity (TIA, mg/g) in sesame seeds\*.

Treatment	Phytic acid	Loss (%)	Tannins	Loss (%)	TIA	Loss (%)
<b>Brown sesame</b>						
Raw seeds	6.5 ± 0.05 a		1.8 ± 0.04 a		1.16 ± 0.07 a	
Roasting	5.0 ± 0.17 b	23.1	1.5 ± 0.08 b	16.6	0.45 ± 0.10 b	61.2
<b>White sesame</b>						
Raw seeds	4.2 ± 0.14 c		2.7 ± 0.08 c		0.73 ± 0.08 c	
Roasting	3.0 ± 0.10 d	28.6	1.7 ± 0.07 a	37.0	0.50 ± 0.02 b	31.5
<b>Tehineh</b>	3.7 ± 0.04 e		1.8 ± 0.03 a		0.20 ± 0.09 d	

\* Mean values of each column followed by different letter are significantly different at  $p < 0.05$ .

other plant foodstuff (Habiba, 2002; Fagbemi *et al.*, 2005; Frontela *et al.*, 2008; Wang *et al.*, 2008). The apparent decrease in phytate content during thermal processing may be partly due either to the formation of insoluble complexes between phytate and other components, such as phytate-protein and phytate-protein-mineral complexes or to the inositol hexaphosphate hydrolyzed to penta- and tetraphosphate (Siddhuraju and Becker, 2001). On the other hand, some authors reported that phytic acid contents were unaffected or increased after heat treatments (Yagoup and Abdalla, 2007; Martín-Cabrejas *et al.*, 2009; Embaby, 2010).

*Effect of heat treatments on Tannins* In peanut, most tannins are located in seed coats (skin) and hulls while fruit (meat nut) are practically tannin free (Shahidi and Nazck, 2004). In the present study, tannins were determined in the raw and processed peanut seeds without removal the skin. This could explain the higher levels of tannins (8.9 mg/g) in peanut seeds (Table 1). The application of all heat treatments, in the present study, significantly ( $p < 0.05$ ) reduced the level of tannins. Reductions in tannins contents ranged from 6.7% to 68.5% in boiled, microwave cooked and autoclaved peanut seeds (Table 1) and ranged from 15.7% to 36.0% in roasted peanut seeds (Table 2). Autoclaving either for 10 or 20 min was the most effective for tannins reduction (reductions were 57.3 and 68.5%, respectively). The levels of tannins in raw and processed sesame seeds are shown in Table 3. Both brown and white raw sesame seeds contained low levels of tannins (1.8 and 2.7 mg/g, respectively). Roasting significantly ( $p < 0.05$ ) reduced the tannins contents in both brown (by 16.6%) and white (by 37.0%) sesame seeds. Also, Tehineh contained a low level of tannins (1.8 mg/g). The reductions in tannins contents in either peanut or sesame seeds agree with the earlier investigations in boiled, microwave cooked, autoclaved and roasted plant foodstuff (Habiba, 2002; Fagbemi *et al.*, 2005; Nithya *et al.*, 2007). The reduction in tannins contents during roasting treatments might be due to the loss of compounds while treating at a high temperature (Nithya *et al.*, 2007). Also, the loss of tannins may be due to the degradation or interaction with other components of seeds, such as proteins, to form insoluble complexes (Embaby, 2010). In contrast to our results, Osman (2007) reported a significant increase in tannins contents in cooked, autoclaved and roasted Dolichos lablab bean. Additionally, Embaby (2010) found that autoclaving, ordinary cooking and microwave cooking didn't affect tannins contents in bitter lupin seeds.

*Effect of heat treatments on trypsin inhibitor activity (TIA)* The presence of protease inhibitors in the diet leads to the formation of the irreversible trypsin enzyme-trypsin inhibitor complex, causing a trypsin drop in the intestine and

a decrease in the diet protein digestibility, leading to slower growth. Under this situation, the organism increases the secretory activity of the pancreas, which could cause pancreatic hypertrophy and hyperplasia (Liener, 1994). The results of TIA in raw (5.6 mg/g) and processed peanut seeds are given in tables 1 and 2. A major beneficial effect of heat treatments of different seeds is the destruction of protease inhibitors, which interfere in protein digestibility. Significant reductions of trypsin inhibitor activities have been noticed after all heat treatments in peanut seeds. Moreover, the longer time of both boiling (for 40 min) and autoclaving (for 20 min) caused a complete inactivation of TIA, but the longer time of microwave (12 min) reduced TIA by 61.5% (Table 1). Between roasting treatments, oil-roasting and roasting-salting were the most effective for trypsin inhibitor inactivation (reductions were 88.6% and 90.4% respectively) (Table 2). Although, oil-roasting was performed in a lower temperature (140°C) it considerably decreased TIA and this could be attributed to the faster and more efficient heat transfer (into the core of the kernels) by oil-roasting than other dry roasting treatments. Also, during the roasting-salting treatment, the salt enhanced the protein (trypsin inhibitor) denaturation, leading to a higher inactivation of TIA. Conclusively the trypsin inhibitor of the studied peanut seeds was heat-labile and moist heat treatments (boiling, microwave and autoclaving) were more effective than roasting treatments for trypsin inhibitor inactivation. Table 3 shows trypsin inhibitor activities in raw and processed sesame seeds. Both brown and white raw sesame seeds had low levels of TIA (1.16 and 0.73 mg/g, respectively). Roasting in both brown and white sesame seeds caused a significant reduction ( $p < 0.05$ ) in TIA (reductions were 61.2 and 31.5 %, respectively). Compared to the raw and processed sesame seeds, Tehineh contained the lowest level of TIA (0.20 mg/g). Similar results of partial inactivation of trypsin inhibitor activity were reported by other workers in roasted legumes (Fagbemi *et al.*, 2005; Osman, 2007). Also, partial and complete inactivation of trypsin inhibitor activity was reported by others in cooked, autoclaved and microwave cooked legumes (Siddhuraju and Becker, 2001; Habiba, 2002; Wang *et al.*, 2008; Martín-Cabrejas *et al.*, 2009; Embaby, 2010). Reactions involving deamidation splitting of covalent bonds, such as hydrolysis of peptide bonds at aspartic acid residues, and interchange or destruction of disulfide bonds, might be involved in the thermal inactivation (Alonso *et al.*, 1998).

*Effect of heat treatments on lectin activity* The evaluation of lectin in both peanut and sesame seeds samples was carried out by using the hemagglutination assay. The estimation of lectin content by hemagglutination of red cells is not a very precise method although the procedure used

is a safe method for checking the efficiency of treatments with respect to seed toxicity (Grant *et al.*, 1982). According to Lis and Sharon (1986) erythroagglutination by lectin is affected by the molecular properties of lectin, cell surface properties, metabolic state of cells and conditions of assay, such as temperature, cell concentration and mixing. Hemagglutinating activities in raw and processed peanut seeds are shown in Table 4. The lectin (from peanut) did not agglutinate untreated or trypsin-treated human erythrocytes whether type A, B, or O, however, after treatment of the erythrocyte with neuraminidase all the above erythrocytes were highly susceptible to agglutination. The hemagglutinating activity was very high in raw peanut seeds (1024, 2048 and 2048 units for type A, B and O, respectively). Fortunately, some of the studied heat treatments (boiling, autoclaving, oil-roasting and roasting-salting) totally destroyed the peanut lectin. Also, hemagglutinating activity was markedly reduced when

subjected to other heat treatments (microwave, roasting and roasting in-shell). This showed that hemagglutinin of the studied peanut seeds was heat-labile. Table 5 shows results for hemagglutinating activity (using 3% albino rat red blood cells) in raw and processed sesame seeds. The hemagglutinating activity in both raw white and brown sesame seeds was low (16 units). Roasting in white and brown sesame seeds caused a significant, but not completely, reduction in hemagglutinating activity (the reduction was 50%). Also, Tehineh contained a low level of hemagglutinating activity (8 units). These results are in good agreement with the previous findings of some investigators in other seeds (Habiba, 2002; Martín-Cabrejas *et al.*, 2009; Embaby, 2010) who reported that lectin activity had completely or partially disappeared after heating (moist or dry heating). The elimination/reduction of lectin activity may be due to the breakdown of hemagglutinins (proteins) into their subunits or undergoing

**Table 4.** Effect of heat treatments on hemagglutinating activity (Units) and *in vitro* protein digestibility (IVPD, %) in peanut seeds\*.

Treatment	Lectin						IVPD	Increase (%)
	A	Loss (%)	B	Loss (%)	O	Loss (%)		
<b>Raw seed</b>	1024		2048		2048		73.4 ± 0.51 a	
<b>Boiling</b>								
20 min	2	99.8	0.00	100	0.00	100	82.8 ± 0.65 b	12.8
40 min	0.00	100	0.00	100	0.00	100	84.9 ± 0.45 c	15.7
<b>Microwave</b>								
6 min	256	75	512	75	256	87.5	78.6 ± 0.47 d	7.1
12 min	16	98.4	16	99.2	8	99.6	79.7 ± 0.54 e	8.6
<b>Autoclaving</b>								
10 min	0.00	100	0.00	100	0.00	100	83.9 ± 0.29 f	14.3
20 min	0.00	100	0.00	100	0.00	100	84.7 ± 0.36 c	15.4
<b>Roasting</b>								
Shelled	8	99.2	8	99.6	4		81.7 ± 0.41 g	11.3
Roasting+Salting	0.00	100	0.00	100	0.00	100	80.9 ± 0.80 g	10.2
Inshell	16	98.4	16	99.2	8	99.6	81.6 ± 0.54 g	11.2
Oil-roasting	0.00	100	0.00	100	0.00	100	84.8 ± 0.36 c	15.5

\* Mean values of each column followed by different letter are significantly different at  $p < 0.05$ .

**Table 5.** Hemagglutinating activity (Units) and *in vitro* protein digestibility (IVPD, %) in sesame seeds and tehineh\*.

Treatment	Lectin	Loss (%)	IVPD	Increase (%)
<b>Brown sesame</b>				
Raw	16		77.9 ± 0.95 a	
Roasting	8	50	85.7 ± 0.46 b	10
<b>White sesame</b>				
Raw	16		77.9 ± 0.91 a	
Roasting	8	50	85.0 ± 0.54 b	9.1
<b>Tehineh</b>	8		82.8 ± 0.81 c	

\* Mean values of each column followed by different letter are significantly different at  $p < 0.05$ .

some other unknown conformational changes in their native structure which might be required for their hemagglutinating activity (Batra, 1987).

*Effect of heat treatments on protein digestibility* Protein digestibility is a primary determinant of the availability of amino acids and, therefore, protein digestibility is important in evaluating the nutritive quality of a food protein. *In vitro* protein digestibility (IVPD) of raw peanut and raw sesame seeds are shown in Tables 4 and 5. Raw peanut and raw sesame seeds (brown and white) exhibit 73.4%, 77.9% and 77.9% IVPD, respectively, which is close to that of many common legumes. Compared with raw peanut seeds, all heat treatments exhibited a significant improvement of IVPD. The increases of protein digestibility produced by boiling, microwave and autoclaving ranged from 7.1 to 15.7% and ranged from 10.2 to 15.5% for roasting treatments. Moreover, boiling for 40 min, autoclaving for 20 min and oil-roasting are the most effective for improving IVPD (the increases were 15.7, 15.4 and 15.5%, respectively). For sesame seeds, roasting significantly improved IVPD in both brown (by 10%) and white (by 9.1%) seeds. Also, Tehineh exhibited a high IVPD (82.8%). Our results agree with those found by other workers (Habiba, 2002; Fagbemi *et al.*, 2005; Embaby, 2010) in roasted, cooked, autoclaved and microwave cooked legume seeds. However, Osman (2007) and Yagoub and Abdalla (2007) found that roasting, autoclaving and cooking significantly decreased IVPD in Dicholas lablab seeds and bambara groundnut, respectively.

Improvement of protein digestibility after processing could be attributed to the reduction or elimination of different antinutrients. Phytic acid, as well as condensed tannins and polyphenols are known to interact with protein to form complexes. These interactions could increase the degree of cross-linking, decreasing the solubility of proteins making protein complexes which impair protease access to labile peptide bonds (Genovese and Lajolo, 1996). In addition, thermal processing promoted structural changes of protein such as globulin, thereby increasing chain flexibility and accessibility to proteases (Swaigood and Catignani, 1991).

## Conclusion

In conclusion, peanut seeds contained high levels of the studied antinutritional factors but they did not contain  $\alpha$ -amylase inhibitor. In this work boiling, microwave, autoclaving and roasting were compared as to their efficiency in reducing antinutrients levels and improving IVPD in peanut seeds. All heat treatments (boiling, microwave, autoclaving and roasting) resulted in a reduction in all studied antinutritional factors and improved the IVPD. Autoclaving, boiling, roasting-salting and oil-roasting were the most effective in

reducing the levels of antinutritional factors and improving IVPD. Also, the results indicated that both brown and white sesame seeds contained tannins, trypsin inhibitor, phytic acid and lectin but  $\alpha$ -amylase inhibitor was absent. Roasting in both brown and white sesame seeds partially eliminated all antinutritional factors and improved IVPD. Moreover, Tehineh contained low levels of the studied antinutrients and exhibited a high IVPD.

## References

- Abu-Jdayil, B., Al-Malah, K. and Asoud, H. (2002). Rheological characterization of milled sesame (teherine). *Food Hydrocolloids.*, **16**, 55-61.
- Alonso, R., Orue, E. and Marzo, F. (1998). Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. *Food Chem.*, **63**, 505-512.
- AOAC (1990). Official Method of Analysis of the Association Official Analytical Chemists, 15<sup>th</sup> ed. (Helrich K., ed.) Arlington, VA.
- Batra, V.I.P. (1987). Effects of cooking and germination on haemagglutinating activity in lentil. *Indian J. Nutr. Diet.*, **24**, 15-19.
- Chukwumah, Y., Walker, L., Vogler, B. and Verghese, M. (2007). Changes in the phytochemical composition and profile of raw, boiled, and roasted peanuts. *J. Agric. Food Chem.*, **55**, 9266-9273.
- Ejigui, J., Savoie, L., Marin, J. and Desrosiers, T. (2005). Influence of traditional processing methods on the nutritional composition and antinutritional factors of red peanuts (*Arachis hypogea*) and small red kidney beans (*Phaseolus vulgaris*). *J. Biol. Sci.*, **5**, 597-605.
- Embaby, H.E. (2010). Effect of soaking, dehulling and cooking methods on certain antinutrients and *in vitro* protein digestibility of bitter and sweet lupin seeds. *Food Sci. Biotechnol.*, **19**, 1055-1062.
- Fagbemi, T.N., Oshodi, A.A. and Ipinmoroti, K.O. (2005). Processing effects on some antinutritional factors and *In vitro* multienzyme protein digestibility (IVPD) of three tropical seeds: Breadnut (*Artocarpus altilis*), Cashewnut (*Anacardium occidentale*) and Fluted Pumpkin (*Telfairia occidentalis*). *Pakistan J. Nutr.*, **4**, 250-256.
- Francis, G., Makkar, H.P.S. and Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, **199**, 197-227.
- Frontela, C., Garcia-Alonso, F.J., Ros, G. and Martinez, C. (2008). Phytic acid and inositol phosphates in raw flours and infant cereals: The effect of processing. *J. Food Compos. Anal.*, **21**, 343-350.
- Genovese, M.I. and Lajolo, F.M. (1996). *In vitro* digestibility of albumin proteins from *Phaseolus vulgaris* (L.). Effect of chemical modification. *J. Agric. Food Chem.*, **44**, 3022-3028.
- Grant, G., More, L.J., McKenzie, N.H. and Pusztai, A. (1982). The

- effect of heating on the haemagglutination activity and nutritional properties of bean (*Phaseolus vulgaris*) seeds. *J. Sci. Food Agric.*, **33**, 1324-1326.
- Grant, G. (1991). Lectins. In: "Toxic Substances in Crop Plants" ed. by F.J.P. D'Mello, C.M. Duffus and J.H.Z. Duffus. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge CB4 4WF, Cambridge, pp. 49-67.
- Habiba, R.A. (2002). Changes in anti-nutrients, protein solubility, digestibility, and HCl-extractability of ash and phosphorus in vegetable peas as affected by cooking methods. *Food Chem.*, **77**, 187-192.
- Hamerstrand, G.E., Black, L.T. and Glover, J.D. (1981). Trypsin inhibitors in soy products: Modification of the standard analytical procedure. *Cereal Chem.*, **58**, 42-45.
- Hsu, H.W., Vavak, D.L., Saterlee, L.D. and Miller, G.A. (1977). Multi-enzyme technique for estimating protein digestibility. *J. Food Sci.*, **42**, 1269-1273.
- Kakade, M., Rackis, J.J., McGhee, J.E. and Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. *Cereal Chem.*, **51**, 376-382.
- Kanu, P.J., Zhu, K., Kanu, J.B., Zhou, H., Qian, H. and Zhu, K. (2007). Biologically active components and nutraceuticals in sesame and related products: a review and prospect. *Trends Food Sci. Technol.*, **18**, 599-608.
- Kortt, A.A. (1984). Purification and properties of the basic lectins from winged bean seed [*Phosphocarpus tetragonolobus* (L) D. C.]. *European J. Biochem.*, **138**, 519.
- Latta, M. and Eskin, M. (1980). Simple and rapid colorimetric method for phytate determination. *J. Agric. Food Chem.*, **28**, 1313-1315.
- Liener, I.E. (1994). Implication of antinutritional components in soybean foods. *Crit. Rev. Food Sci. Nut.*, **34**, 31-67.
- Lis, H. and Sharon, N. (1986). Biological properties of lectins. In: The "Lectins: Properties, Functions and Applications in Biology and Medicine" ed. by I.E. Liener, N. Sharon and I.J. Goldstein. Academic Press, Orlando, pp 265-291.
- Lott, J.N.A., Ockenden, I., Raboy, V. and Batten, G.D. (2000). Phytic acid and phosphorus in crop seeds and fruits: a global estimate. *Seed Sci. Res.*, **10**, 11-33.
- Martín-Cabrejas, M.A., Aguilera, Y., Pedrosa, M.M., Cuadrado, C., Hernandez, T., Diaz, S. and Esteban, R.M. (2009). The impact of dehydration process on antinutrients and protein digestibility of some legume flours. *Food Chem.*, **114**, 1063-1068.
- Nestares, T., Barrionuevo, M., Urbano, G. and Lopez-Frias, M. (1999). Effect of processing methods on the calcium, phosphorus and phytic acid contents and nutritive utilization of chickpea (*Cicer arietinum* L.). *J. Agric. Food Chem.*, **47**, 2807-2812.
- Nithya, K.S., Ramachandramurty, B. and Krishnamoorthy, V.V. (2007). Effect of processing methods on nutritional and anti-nutritional qualities of hybrid (COHCU-8) and traditional (CO7) pearl millet varieties in India. *J. Biol. Sci.*, **7**, 643-647.
- Osman, M.A. (2007). Effect of different processing methods, on nutrient composition, antinutritional factors, and *in vitro* protein digestibility of Dolichos lablab bean [*Lablab purpureus* (L) Sweet]. *Pakistan J. Nutr.*, **6**, 299-303.
- Reddy, N.R. (2002). Occurrence, distribution, content and dietary intake of phytate. In: "Food Phytate" ed. By N.R. Reddy and S.K. Sathe. CRC Press, Boca Raton, pp. 25-51.
- Shahidi, F. (1997). Beneficial health effects and drawbacks of antinutrients and phytochemicals in foods. In: "Antinutrients and Phytochemicals in Food" ed. By F. Shahidi. Washington, DC: American Chemists Society, pp. 1-10.
- Shahidi, F. and Naczki, M. (2004). Phenolics in food and nutraceuticals. CRC Press, pp. 17-82.
- Siddhuraju, P. and Becker, K. (2001). Effect of various domestic processing methods on antinutrients and *in vitro*-protein and starch digestibility of two indigenous varieties of Indian pulses, *Mucuna pruriens* var *utilis*. *J. Agric. Food Chem.*, **49**, 3058-3067.
- Swaigood, E. and Catignani, L.G. (1991). Protein digestibility: *In vitro* methods of assessment. In: "Advances in Food and Nutrition Research" ed. by J.E. Kinsella. San Diego: Academic Press, pp. 185-230.
- Vaintraub, I.A. and Lapteva, N.A. (1988). Colorimetric determination of phytate in unpurified extracts of seed and the products of their processing. *Analytical Biochem.*, **17**, 227-230.
- Wang, N., Hatcher, D.W., and Gawalko, E.J. (2008). Effect of variety and processing on nutrients and certain anti-nutrients in field peas (*Pisum sativum*). *Food Chem.*, **111**, 132-138.
- Yagoub, A.A. and Abdalla, A.A. (2007). Effect of domestic processing methods on chemical composition, *in vitro* digestibility of protein and starch and functional properties of bambara groundnut (*Voandzeia subterranea*) seed. *Research J. Agric. Biol. Sci.*, **3**, 24-34.