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EFFECT OF MICROWAVE AND γ- IRRADIATION ON SOME ANTINUTRITIONAL FACTORS IN SOME LEGUME SEEDS

Mahmoud, Magda E.; M. A. Issa; H.S. Abdel-Salam; M. Sh. Hassan and Amal A. Salah

Agric. Chem. Dept., Faculty of Agric., Minia Univ., Minia, Egypt

ABSTRACT

The effect of microwave heating and γ- irradiation treatment are investigated on oligosaccharides, trypsin inhibitor, phytic acid, polyphenols and saponin content of two varieties of two Egyptian legume seeds soybean and kidney bean.

Sucrose constituted 3.76 and 1.94% for dried soybean and kidney been seeds, respectively. The stachyose levels varied from 0.97% for soybean to 1.65% for kidney bean. Soybean seeds contained 0.75% of raffinose versus 0.95% in kidney bean seeds.

Thermal heating for 80 sec of pre-soaked seeds (for 12 hr) produced a sharp reduction in raffinose by 94.67% and stachyose by 92.78% in soybean and 94.74 and 56.36% in kidney bean, respectively. It is noted that the same treatment decreased the sucrose content by 70.21% in soybean and 58.76% in kidney bean.

On irradiation treatment of soya and kidney bean seeds followed by soaking for 6 hr, sucrose content was reduced by 51.86 and 35.57% at 2.5 KGy, and 56.91 and 38.66% at 5.5 KGy of its amount in raw seeds, respectively.

Quantitative measurement indicated that phytic acid was ranged from 3.68 mg/g soybean to 3.25 mg/g kidney bean (defatted meal). All processing decreased phytic acid content in both legume seeds and such decrease increases as soaking and microwave heating period increases .

The maximum decrease in phytic acid was about 70% in soybean and 60% in kidney bean seeds when the soaked seeds exposure to microwave heating for 160 sec. Irradiation at a dose of 7.5 KGy followed by soaking for 12hr resulted in a decrease in phytic acid level in soybean and kidney bean seeds only by 53.8 and 74.77%, respectively.

Trypsin inhibitor activity (TIA) was decreased with increasing microwave-heating period in all treatments. After exposure the presoaked seeds (12 hr) to microwave heating for 160 sec, about 61.80% and 60.25% of the TIA was destroyed in soya and kidney beans respectively. Microwave heating for 160 sec of pre-soaked soya and Kidney bean seeds for 1hr destroyed TIA by 27.8 and 29.5%, respectively. Whereas, TIA degradation in pre-soaked seeds for 6hr was 55.4 and 41.45%. Soaking of irradiated (at 7.5 KGy) soya and kidney bean seeds for 12 hr was found to remove, about 49.8% and 60% of TIA, respectively as compared to the levels in the raw flour, while in other treatments, considerable amounts of trypsin inhibitors well still found.

The obtained results showed a rapid increase in polyphenols content as a result of microwave treatment for 160 sec of soaked seeds for 6hr to reach 27.91% and 33.33% of its amount in raw seeds of soya bean and kidney beans, respectively. Soaking for 12hr followed by microwave heating for 160 sec showed the highest increase of tannins (34.88%) in soybean and (45.33%) in kidney bean. While, irradiation at dose 7.5 KGy accompanied by soaking for 12hr raised the content of polyphenols content in soybean and kidney beans by 1.4 and 1.7-fold, respectively.

The results showed that eight olive green to purple (mauve) spots have been detected in raw soybean seeds. The spots corresponding to glycosides with R_f values of 0.06, 0.19, 0.25 and 0.44 were having high intensity. On the other hand, the

y of the spots with R_f of 0.50 and 0.65 were moderate and those of R_f values ıd 0.90 were low. Also, crude saponin of raw kidney bean seeds gave only four components with the corresponding values of R_f 0.50, 0.65, 0.81 and 0.90.

TLC examination of crude saponin extracted from microwave heated (160 sec) of pre-soaked (12hr) soybean and kidney bean seeds showed 6 and 2 spots, respectively. In contrast, TLC of crude saponin patterns showed no detectable differences between non-irradiated and irradiated (at all doses) pre-soaked legume seeds.

INTRODUCTION

Legumes are of especial significance as a plant food source since they are high protein crops. On average, cereal grains contain 10-15% of the dry weight as protein, whereas legume seeds contain 20-30% and up to 50% of the dry weight as protein in some varieties of soybean (*Glycine max*). In contrast, a typical vegetative organ, such as leaf, has only 3-5% of its dry matter as protein. Some legume seeds are also rich in oil, concentrations vary from 1% to more than 40%. Although relatively poor in some vitamins, such as retinol, riboflavin and ascorbic acid, legumes have reasonable quantities of thiamin and nicotinic acid. The nutritionally important minerals, calcium and iron are also present as well as fiber. Legume fiber consists of polysaccharides and lignins that resist hydrolysis by human digestive enzymes and form viscous solutions or gells with water. This fiber is of particular importance medically due to its component seed storage galactomannans, important in the treatment of diabetes. Also, this fiber may have hypoglycemic and hypo cholesterolemic effects and may reduce risks of colon cancer (Anderson *et al*, 1990). Dry beans have beneficial effects on human health, being very low in sodium (Cheftel *et al*, 1986), cholesterol (Luc *et al*., 1991), and saturated fatty acids but rich in unsaturated fatty acids such as linoleic acid (Besancon, 1978). In the final analysis the nutritional importance of a seed constituent must be related to the purpose for which the seed meal is being used e.g. animal feed, human food etc. either directly or more recently, as textured vegetable protein foods.

Despite such advantages, legumes present some undesirable characteristic which limit their acceptability, these include the hard-to-cook phenomenon (Salunkhe and Kadam 1989), the presence of oligosaccharides causing flatulence, antinutritional factors and low protein digestibility. In order to improve the nutritional quality of legumes, treatments such as soaking, cooking, germination, supplementation, microwave heating or irradiation have been applied.

Since, recently microwave heating is one of the preferred methods to improve the nutritional quality of legumes because of the speed and minimal loss of chemical alteration. Also, the use of irradiation technology is promising owing to the minimal effect if suitable doses are applied. Also, radiation decontamination is technically feasible, economically viable and safe physical process and leavens no toxic residues.

In order to improve the nutritional quality of dry bean, treatments such as soaking, cooking, germination, microwave exposure or γ-irradiation have bean applied (Vishalakshi *et al*, 1980 and El-Nahry *et al*, 1977).

The purpose of this investigation is to study the effect of microwave treatments and gamma-irradiation on oligosaccharides and antinutritional factors, trypsin inhibitor, phytic acid, polyphenolic compounds, and saponin in soybean (variety Giza-35) and kidney bean (variety Paullista).

MATERIALS AND METHODS

Source of samples:

The seeds of soybean (*Glycine max*) variety Giza-35 and Kideny bean (*Phaseolus vulgaris*) variety Paullista were obtained from Agricultural Research Centre, Giza, Egypt.

Processing of samples:

The normal procedures used for preparing legume seeds were used.

Heat treatment:

Water- soaked seeds were prepared by adding 500g of whole dry seed of both legumes to 1500 ml of distilled water and allowing them to imbibe water 20 ^{0}C for various intervals as follows:

1- Air-dried seeds (control).
2- Water-soaked seeds (for 1 hr).
3- Water-soaked seeds (for 6 hr).
4- Water soaked seeds (for 12 hr).

Water-soaked seeds (50g + 50 ml H_2O) were heated in the microwave oven for 80,120 and 160 seconds. Goldstar microwave (Fr. 2450 MHz- input 220 v 50 Hz, 980 W) was used. To obtain uniform heating, a turlable was installed which slowly rotated the sample in a direction opposite the built-in microwave stirrer. The microwave-heated samples were air-dried over night at room temperature and then stored in closed container at 4 ^{0}C until used.

Irradiation treatment:

Seeds uniform size were packed in polyethylene bags and exposed at 28 ± 2 ^{0}C to ^{60}Co gamma radiation at doses of 2.5, 5.5 and 7.5 KGy, in the Middle Eastern Region Radioisotopes Center for the Arab Countries, Cairo, Egypt. Irradiated samples were stored for 4 weeks (to eliminate the irradiation-induced damage) and then soaked in water (500g seed in 1500 ml water) for several times as follows at 20 ^{0}C:

Soaking in water for 1 hr.
Soaking in water for 6 hr.
Soaking in water for 12 hr.

Preparation of defatted meal:

All treated samples were grounded in coffee grinder and defatted using ice-cold acetone in a blender. The defatted matter (acetone powder) was air-dried at room temperature and stored in a closed container at 4 ^{0}C until required.

Determination of raffinose oligosaccharides:

Oligosaccharides were extracted from powdered samples with 80%(v/v) ethanol (Akpapunam and Markakis, 1979). Unidirectional descending paper (whatman No.1, 20 x 45 cm) chromatography using a

solvent of n- butanol, ethanol and water (5:3:2 by volume) was conducted for 48 hr. to separate oligosaccharides. The sugars were identified on the basis of their R_F and R_G values according to (Akpapunam and Markakis, 1979). The concentration of the identified sugars was determined using the phenol sulphoric acid method of Dubois *et al.*, (1956).

Extraction and determination of total phenolic compounds (TPC)

The total phenolic compounds (TPC) were extracted from each defatted sample (500 mg) by refluxing with 50 ml of methanol containing 1 % HCl for 20 min. The extract was centrifuged at 10000 r.p.m. for 20 min. After leaving over night at 4°C, the solution was centrifuged at 10000 r.p.m. for 15 min.. The amount of phenolic compounds was estimated as tannic acid equivalent according to the Folin-Denis procedure (Swain and Hills, 1959).

Determination of phytic acid:

Phytic acid was determined according to the calorimetric method of Hang and Lantzsch (1983).

Extraction of trypsin inhibitor (TI) :

Finely ground defatted sample was suspended in distilled water at ratio of 1 :10 (w/v) and stirred for one hr at room temperature. The suspended obtained after centrifugation at 10000 r.p.m. for 30 min. was used for assaying trypsin inhibitor activity.

Determination of trypsin inhibitor (TIA) :

TIA was measured spectrophotometrically using N^{α} -Benzoly-D L-arginie-p-nitroanilide H Cl (BAPNA) a s a s ynthetic s ubstrate f or t rypsin as described by Hamerstrand et al., (1981) with modification with respect to the initiation of the TIA assay, i.e. trypsin was added as the last component to the inhibitor-substrate mixture (Staufer, 1993).

Extraction of saponins:

Air-dried powdered of treated seed samples (100 g), were extracted during 5 days at room temperature with 650 ml of ethanol. The solvent was evaporated and the residue suspended in H_2O (60 ml). This suspension was partitioned first with dichloromethane (165 ml) and then with n-butanol (250 ml). The alcoholic phase was concentrated yielding the crude saponins mixture (Pires *et al.*, 1997).

Thin layer chromatography (TLC) of crude saponins:

Standard sampels (5-50 µl) of ethanolic saponin extracts were spotted on pre-coated silica gel TLC plates (Merck, kieselgel 60 F-254). The plates were developed with n-butanol: ethanol: conc. ammonia (2.5: 1: 2.5). To visualize spots, the plates were sprayed with 100 ml / L solution of sulfuric acid i n ethanol and heated at 110 °C for 30 min., two different procedures were used:

1-A chromatogram was sprayed with a mixture of *P*-anisaldhyde(1ml), sulfuric acid (2 ml) and acetic acid (98ml) and heated for1 min.at 100 °C. The reagent gives characteristic purple- mauve color with steroids or triterpenes (Lisboa, 1976).

2-A chromatogram was dipped for 1 min. into a s olution c ontaining 2 .7 M aqueous silver nitrate (2 ml) in acetone (400 ml). It was then dried and sprayed with 0.5M sodium hydroxide in ethanol. Reducing sugars give a dark brown coloration and a light background (Trevelyan et al., 1950).

RESULTS AND DISCUSSION

Oligosaccharide content :

The oligosaccharide content of the whole raw seeds showed that the concentration of sucrose, compared with other oligosaccharide was the highest in both legumes followed by stachyose then raffinose, (Table 1).

Sucrose constituted 3.76 and 1.94% for soybean and kidney bean, respectively. The stachyose levels varied from 0.97% for soybean to 1.65% for kidney bean. Fore raffinose, kidney bean contained 0.95% while soybean contained 0.75% (Table 1).

Our data of oligosaccharides in soybean (Table 1) are very close to that findings of Abdel-Hakim (1998) who found the amounts of sucrose, raffinose and stachyose varied from 3.63 to 3.88%; from 0.5 to 0.6% and from 0.94 to 1.14%, respectively.

Table (1): Effect of microwave treatment on oligosaccharide contents in pre-soaked two legume seeds (% on dry weight basis).

Treatment	Sucrose		Raffinose		Stachyose	
	Soya bean	Kidney bean	Soya bean	Kidney bean	Soya bean	Kidney bean
Raw Seeds	3.76	1.94	0.75	0.95	0.97	1.65
Time of exposure (sec)	Soaking for 1 hr					
0	2.72	1.84	0.66	0.82	0.74	1.52
80	2.46	1.75	0.54	0.73	0.64	1.42
120	2.25	1.66	0.45	0.7	0.55	1.35
160	2	1.56	0.37	0.67	0.3	1.31
	Soaking for 6 hr					
0	1.92	1.33	0.32	0.66	0.27	1.25
80	1.8	1.28	0.25	0.57	0.23	1.19
120	1.72	1.21	0.2	0.5	0.2	1.1
160	1.61	1.15	0.18	0.44	0.17	1
	Soaking for 12 hr					
0	1.35	0.79	0.07	0.22	0.1	0.9
80	1.12	0.8	0.04	0.05	0.07	0.72
120	0.95	0.72	0	0	0.04	0.65
160	0.76	0.61	0	0	0.02	0.33

Production of flatulence in mammals is closely associated with the consumption of legumes, specially dry bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) through the gas-forming quality which develops during maturation of seeds, differs in concentrations depending upon their species, variety and growth conditions (Calloway *et al*, 1971).

Low molecular weight oligosaccharides of the raffinose family, specially raffinose and stachyose are shown to be primary agents responsible for flatulence activity in mature seeds. Non-reducing sugars cannot be completely metabolized due to the absence of α – 1,6 – galactosidase (α-*D*-

galactosidase, galactohydrolase E.C. 3-2-1-22) activity in mammalian intestinal mucosa. Consequently, anaerobic bacteria in lower intestinal tract degrade them to yield gases such as hydrogen, carbon dioxide and small amounts of methane (Rackis 1981, as well as Salunkhe, 1982).

In order to improve the nutritional quality of dry bean, treatments such as soaking, cooking, germination, microwave exposure or γ-irradiation have bean applied (Vishalakshi *et al*, 1980 and El-Nahry *et al*, 1977).

Table (1) illustrated the variation in oligosaccharides as a result of microwave treatments. It is obvious that all treatments reduced sucrose and raffinose oligosaccharides (raffinose, stachyose). Thermal heating for 80 sec of pre-soaked seeds (for 12 hr) produced a sharp reduction in raffinose by 94.67% and stachyose by 92.78% in soybean and 94.74 and 56.36% in kidney bean, respectively. Prolonged time of exposure for 160 sec c aused disappearance of raffinose in both legumes and reduction of stachyose by 97.94% in soybean and 80% in kidney bean.

It is noted that the same treatment decreased the sucrose content by 79.79% in soybean and 68.56% in kidney bean, (Table 1).

We can say that the greatest decrease of flatulent factors was achieved by microwave heating for 160 sec after soaking the raw seeds for 12 hr.The decrease may due to a loss of sugar to water by diffusion and or by enzymatic degradation in microwave treatment.

Raffinose oligosaccharides reduction has been reported in soaked or soaked cooked beans by Barampama and Simard, (1994) in red bean and horse bean (Jood *et al*, 1985).

On irradiation treatment of soya and kidney bean seeds followed by soaking for 6 hr, sucrose content was reduced by 51.86 and 35.57% at 2.5 KGy and 56.91 and 38.66% at 5.5 KGy, respectively (Table 2).

The obtained data; (Table 2) showed that stachyose and raffinose were broken down much faster. The raffinose disappeared completely in the soaked soybean seeds for 12 hr at 2.5 KGy, while 76.84% of its content was decreased in kidney seeds at the same treatment.

By increasing irradiation dose up to 7.5 KGy raffinose of kidney bean seeds (soaked for 12hr) decreased by 94.74% (Table2). Concerning stachyose irradiation at 7.5 KGy caused decrease of its content in s oaked seeds (for 12 hr) of soybean by 94.85% and 93.94% of kidney bean (Table2).

It is known that removal of stachyose from soybean helps to improve the digestibility of soybean and increase the potential of soybean for human food. So the Egyptian soybean, variety Giza- 35 good nutritional quality, which had low content of stachyose, would exhibit.

The decrease in concentration of stachyose and raffinose might be due to heat hydrolysis of oligosaccharides to simple disaccharides, monosaccharides, or the formation of other compounds.

Our finding were found to be similar in most aspects with those reported by other investigators (Ghazy, 1990; Ismail *et al*, 1995 and Fawzy, 1998).

Generally, fragmentation of stored carbohydrates to low molecular weight sugars in γ-irradiation or microwave treatments may due to direct break of molecule by radiation or by stimulating of hydrolytic activity.

Verbascose could not be detected in raw or treated soya and kidney bean seeds by paper chromatography technique used in this study. This means that verbascose is not absolutely present or present at below detectable level in both raw and treated seeds of two legumes. This finding was disagreed with those by (Darwish, 1998) who found that verbascose composed about 0.9% of dry mature cowpea seeds var. cream-7. Also, Reddy and Pierson (1984) observed that verbascose ranged from 0.1 to 0.5% in red kidney and soya beans seeds. However, the absence of verbascose in some legume seeds has previously been reported (Eldridge, *et al*, 1979; Molhar-Perl and Szakacs-Pinter, 1985; Liu and Markakis, 1987 and Abdel-Hakim, 1998).

Table (2): Effect of soaking on oligosaccharide contents in pre-irradiated two legume seeds (% on dry weight basis).

Treatment	Sucrose		Raffinose		Stachyose	
	Soya bean	Kidney bean	Soya bean	Kidney bean	Soya bean	Kidney bean
Raw Seeds	3.76	1.94	0.75	0.95	0.97	1.65
Irradiation dose (KGy)	Soaking for 1 hr					
0	2.72	1.84	0.66	0.82	0.74	1.52
2.5	2.35	1.66	0.66	0.69	0.61	1.35
5.5	2.22	1.5	0.65	0.61	0.46	1.31
7.5	2.1	1.42	0.62	0.56	0.32	1.26
	Soaking for 6 hr					
0	1.92	1.32	0.35	0.66	0.27	1.25
2.5	1.81	1.25	0.35	0.46	0.27	1.15
5.5	1.62	1.19	0.35	0.4	0.2	0.97
7.5	0.94	1.15	0	0.33	0.15	0.82
	Soaking for 12 hr					
0	1.35	0.97	0.07	0.22	0.1	0.9
2.5	0.85	0.86	0	0.22	0.1	0.64
5.5	0.71	0.67	0	0.15	0.07	0.36
7.5	0.61	0.57	0	0.05	0.05	0.1

Phytic acid:

The content of phytic acid in raw and microwave of pre-soaked two legume seeds is shown in (Table 3). Phytic acid ranged from 3.68 mg/g soybean to 3.25 mg/g kidney bean (defatted meal). The level of phytic acid in the two legumes seemed to be lower than that reported (for soybean, 182mg/g) by Thompson and Erdman (1982) and (for kidney bean seeds, 1.9 and 2.1%) in Belfin and Nomad seeds varieties by Fawzy (1998). But it seemed to be consistent with those reported for soybean, Giza-21and Giza-82 by Issa *et al* (2002).

Data, shown in (Table 3) indicated that all processing decreased phytic acid content in both legume seeds and such decrease increases as soaking and microwave heating period increases. After soaking for 1hr, 6hr, and 12hr the original content of phytic acid (3.68, 3.25 in soya and kideny

bean respectively), had decreased by 3.45, 3.38%; 39.40, 27.38% and 45.11, 47.08% in soya and kidney beans, respectively (Table 3). Similar substantial decreases in phytic acid content have been observed in soaked kidney beans (Lolas and Markakis, 1975); in soybeans (de Boland *et al*, 1975) and in brown beans (Gustafasson and Sandberg, 1995).

Table (3): Effect of microwave treatment on phytic acid content in pre-soaked two legume seeds (% on dry weight basis).

Treatment	Soya bean	Kidney bean	Soya bean	Kidney bean	Soya bean	Kidney bean
Time of exposure (sec)	Soaking for1hr		Soaking for6hr		Soaking for 12hr	
0	3.52	3.14	2.23	2.36	2.02	1.72
80	3.35	3.00	2.19	2.29.	1.56	1.62
120	3.09	2.72	2.15	2.00	1.23	1.45
160	2.94	2.52	2.09	1.82	1.10	1.30

Upon comparing the absolute loss of phytic acid during different processing methods, Tables (3 and 4), it was observed that, soaking for 1hr accompanied by microwave heating for 80, 120 and 160 sec caused a small decrease in phytic acid content in soybean and kidney bean by 8.97, 16.03, 20.11% and 7.69, 16.31 and 22.46% compared with raw legume seeds, respectively. The same microwave treatment on pre-soaked two legume seeds for 6hr resulted in a moderate decrease in phytic acid levels ranging from 40.49 to 43.21% in soybean and 29.54 to 44% in kidney bean, respectively.

The maximum decrease in phytic acid was observed, being about 70.11% in soybean and 60% in kidney bean seeds when soaked seeds (for 12hr) exposure to microwave heating for 160 sec (Table 3). The above observations indicated that soaking in water for 12hr followed by microwave heating for 160 sec was the most effective treatment for lowering phytic acid of both legumes.

As regards the effect of irradiation on phytic acid (Table 4), it is noted that a dose of 7.5 KGy and soaking for 12 hr, caused a decrease in phytic acid of soybean only by 53.80%. This indicates the need for higher irradiation doses. The same dose caused loss of phytic acid in kidney bean by 74.77%.

Also it is evident that the decrease of phytic acid by irradiation was proportional to the dose and soaking time for both legumes.

The obvious decrease in phytic acid of both legumes under consideration could be attributed to leaching of phytate ions into soaking water. Such losses may be taken as a function of changed permeability of seed coat.

Our results are in agreement with those reported by Khan *et al*, (1988) and Hafez *et al*, (1989) as well as Vidal-Valverde *et al*, (1994). Contrarily, the obtained results disagree with the findings of Ologhobo and Fetuga (1984) who indicated that cooking, autoclaving and soaking were not effective in lowering the phytate content of soybean. Also, Barampama and

Simard (1994) reported that soaking or cooking did not reduce phytic acid content of dry beans.

Table (4): Effect of soaking on phytic acid content in pre-irradiated two legume seeds (% on dry weight basis).

Treatment	Soya bean	Kidney bean	Soya bean	Kidney bean	Soya bean	Kidney bean
Irradiation dose (KGy)	Soaking for 1 hr		Soaking for 6hr		Soaking for12 hr	
0	3.52	3.14	2.23	2.36	2.02	1.72
2.5	3.45	2.21	2.62	1.52	2.0 0	1.19
5.5	3.26	2.11	3.55	1.41	1.80	1.00
7.5	3.12	1.86	2.51	1.32	1.70	0.82

Our results were disagreed with those found by Deshpande *et al* (1982) who stated the phytic acid contents of whole beans ranged from 1.16 – 2.93%. In this connection Bassiri and Nahapetian (1977) reported that in many cases phytic acid content is not considered to be absolute and may vary depending upon the variety, and / or cultivar, climatic conditions, location, irrigation conditions, type of soil and year which they are grown.

Trypsin inhibitory activity (TIA):

The effect of microwave treatments and soaking on trypsin inhibitor content of soybean and kidney bean seeds is reported in Table (5)

The obtained data showed that TIA was decreased with increasing microwave heating period in all treatments.

It is observed that after exposure the presoaked seeds (12 hr) to microwave heating for 160 sec, about 61.80% and 60.25% of the TIA was destroyed in soya and kidney beans, respectively.

Table (5): Effect of microwave treatment on trypsin inhibitory activity in pre-soaked two legume seeds (% on dry weight basis).

Treatment	Soya bean	Kidney bean	Soya bean	Kidney bean	Soya bean	Kidney bean
Time of exposure (sec)	Soaking for 1 hr		Soaking for 6 hr		Soaking for 12 hr	
0	87.72	82.4	66.54	65.62	56.82	52.70
80	81.12	79.6.	64.50	61.70	40.90	50.10
120	78.72	74.74	54.40	60.45	39.82	47.11
160	72.20	70.50	44.60	58.55	38.20	39.75

TIA of raw seeds are taken as 100%.

Also, tabulated data indicated that microwave heating for 160 sec of pre-soaked soya and kidney bean seeds for 1hr destroyed TIA by 27.8 and 29.5%, respectively. Whereas, TIA reduction percent in pre-soaked seeds for 6 hr was 55.4 and 41.45%. Thus, loss of TIA after microwave heating from pre-soaked legume seeds increases as heating and soaking period increase.

Prolonging the exposure period make the color of the seeds turned slightly brown. For this reason the 160 sec was taken as optimum for our study.

Our findings were close to that reported by Hewedy (1990) who stated that the increase in soybeans moisture content caused an increase in TI destruction which is highly required to improve the digestibility of soybean proteins. Also, Prinyawiwatkul *et al*, (1996) found that soaking reduced TIA by ≈ 20% whereas boiling of soaked seeds decreased TIA by ≈ 85%.

Soaking of irradiated (at 7.5 KGy) soya and kidney bean seeds for 12 hr was found to remove, about 48.8% and 60% of TIA respectively, as compared to the levels in the raw flour, while in many other treatments, considerable amounts of trypsin inhibitors well still found (Table 6).

Irradiation of pure crystalline soybean trypsin inhibitor by a dose of 100 KGy caused no charge in the activity (Hafez *et al*, 1985), however 98-67% inactivation of trypsin inhibitor was achieved in the aqueous solution.

Table (6): Effect of soaking on trypsin inhibitory activity in pre-irradiated two legume seeds (% on dry weight basis).

Treatment	Soya bean	Kidney bean	Soya bean	Kidney bean	Soya bean	Kidney bean
Irradiation dose(KGy)	Soaking for 1hr		Soaking for 6hr		Soaking for 12hr	
0	87.72	82.40	66.54	65.62	43.82	52.70
2.5	72.77	70.71	60.50	55.45	56.20	51.70
5.5	73.50	68.50	56.70	52.70	52.32	49.72
7.5	71.10	60.59	50.10	51.80	51.20	40.00

Since TIs are low molecular proteins, their extraction from the seed to soaking medium is quite possible. The sodium salt present in the soaking medium is likely to increase the porosity of seed coat. This is, perhaps one of the reasons for greater loss TIA from the seed.

It could be said that the well established heat, either from microwave treatment or irradiation treatment, labile nature of trypsin inhibitors.

Obtained results indicated that the thermal treatments and soaking process do not completely inactivate all trypsin inhibitor in soya and kidney beans. On the other hand, the amount of heat required to destroy TI may destroy lysine and sulfur- containing amino acids and induce browning reactions thus damaging its nutritional quality (Rios-Lriarte and Barnes (1966).

Polyphenols (Tannins):

The content of polyphenols in raw and soaked –microwave-heated soya and kidney bean seeds were collected in (Table 7). The data showed that polyphenols content was increased with increasing of microwave heating time in all cases.

The tabulated data indicated a rapid increase in polyphenols content as a result of microwave treatment for 160 sec of soaked seeds for 6hr to reach 27.91% and 33.33% of its amount in raw seeds 0.86, 0.75 of soya bean and kidney beans, respectively.

Soaking for 12hr followed by microwave heating for 160 sec showed the highest increase of tannins (34.88%) in soybean and (45.33%) in kidney bean.

Our results disagree with those reported by Elias *et al*, (1979) who observed that about 30-40% of polyphenols can be removed from *Phaseolus vulgaris* by cooking and discarding the cooking water solution.

Table (7): Effect of microwave treatment on polyphenols content in pre-soaked two legume seeds (% on dry weight basis).

Treatment	Soya bean	Kidney bean	Soya bean	Kidney bean	Soya bean	Kidney bean
Time of exposure (sec)	Soaking for 1 hr		Soaking for 6hr		Soaking for 12hr	
0	0.86	0.76	0.95	0.84	0.96	0.82
80	0.92	0.76	0.96	0.90	1.00	0.82
120	0.96	0.91	1.00	0.95	1.07	0.95
160	1.07	0.99	1.10	1.00	1.16	1.09

On the other hand, Vidal-Valverde *et al* (1994) reported that cooking of the pre-soaked seeds brought an increase of the content of tannins in lentil (*Lens culinaris* var.vulgaris).

After the soaking process, polyphenols which are originally placed in the interior of the cell could move and being more accessible to subsequent analysis. On the other hand, tannins (polyphenols) can be cross-linked with proteins by reacting with lysine or methionine, carbohydrates, and vitamins forming more or less stable complexes depending on the degree of polymerization. Soaking and microwave heating processes liberate these complexes, therefore the tannin (polyphenols) levels increase.

As regards the influence of soaking of irradiated seeds (2.5-7.5 KGy), data in (Table 8) showed that soaking for 6hr of irradiated (for 7.5 KGy) soya and kidney bean seeds, caused an increase in polyphenols content by 41.86 and 54.67%, respectively.

(Table 8) indicated that the irradiation at dose 7.5 KGy accompanied by soaking for 12hr increased the content of polyphenols in soya and kidney beans by 1.4 and 1.7-fold, respectively.

Table (8): Effect of soaking on polyphenols content in pre-irradiated two legume seeds (% on dry weight basis).

	Kidney bean	Soy bean	Kidney bean	Soy bean	Kidney bean	Soy bean
Irradiation dose (KGy)	Soaking for 1hr		Soaking for 6hr		Soaking for12hr	
0	0.72	0.66	0.65	0.54	0.46	0.32
2.5	0.82	0.7	0.97	0.86	1.09	0.95
5.5	0.97	0.92	1.12	1.07	1.25	1.17
7.5	1.19	1.1	1.22	1.16	1.29	1.2

The increased amount of phenolic compounds observed in irradiated-soaked seeds of soya and kidney beans could be due to the degradation of tannins and consequent higher extractability of phenolic compounds.

Saponins:

Raw and treated two legume seeds were extracted with EtOH. Evaporation of the alcoholic extract furnished a residue that was suspended in H_2O and successively extracted with CH_2Cl_2 and then with n-BuOH. The dried *n*-BuOH-residue (crude saponin) was amorphous, white to pale yellowish white, hygroscopic powder, freely soluble in water, slightly soluble in methanol and ethyl alcohol and insoluble in ether or acetone.

The crude saponin gave positive result with Molische, s test and its aqueous solution produced persistent froth on shaking.

The crude saponin was dissolved in BuOH and subjected to TLC using silica gel (G) and several developing systems were tested. The pest separation was performed with solvent system, BuOH: EtOH: conc. NH_3 (3.5: 1: 2.5 v/v/v). Para-anisaldehyde reagent was used as a developing agent which produce olive green to purple-mauve color after heating at 110 ^{0}C for 10 min.

The obtained results were presented in (Table 9), which showed that eight olive green to purple (mauve) spots have been detected in raw soybean seeds. The spots corresponding to glycosides with R_f values of 0.06, 0.19, 0.25 and 0.44 were having high intensity. On the other hand, the intensity of the spots with R_f of 0.50 and 0.65 were moderate and those of R_f values 0.81 and 0.90 were low.

The purity, origin and structure of the commercial saponin standard were unknown. The saponin standard was visualized as a streak on the TLC plate and therefore may contain a mixture of acidic and natural saponins and non saponin components.

Also, It is noted that saponin of untreated kidney bean seeds (raw)gave only four components with the corresponding values of R_f 0.50, 0.65, 0.81 and 0.90.

Oakenfull (1981) stated that, because differences in the nature of purity of the saponins of standard and samples may have contributed to higher reported saponin contents in comparison with other studies, these values should be used as a mean of comparison between the dry bean classes rather than indication of absolute content

Microwave treatment of pre-soaked seeds in both legumes caused a slight decrease in saponin content since TLC examination showed 6 spots and 2 spots in the crude saponins of soybean and kidney bean, respectively (Figs. 1 and 2).

This slight loss during heat treatment by microwave may be indicating thermolabile nature of saponins. Not much is known about the formation of poorly extractable complex between saponins and sugars or amino acids upon cooking (Khokhar and Chauhan, 1986).

Drumm *et al* (1990) stated that there was a significant decrease in the total saponin content and the content of the three major saponin components (I, III, and IV) as a result of soaking and canning of dry beans.

Similar losses have been noted in the saponin content of moth beans, which have undergone pressure-cooking (Khokhar andChauhan, 1986).

Table (9): Results of TLC examination of the saponins.

Solvent System	R_f			-anisaldehyde *p*
	Standard	Raw		
		Soya	Kidney	
BuOH :EtOH : conc.NH_3 3.5 : 1 : 2.5 (v / v)	0.06	0.06	n.d	Light purple
	0.19	0.19	n.d	Olive green
	0.25	0.25	n.d	Olive green
	0.44	0.44	n.d	Olive green
	0.50	0.50	0.5	Olive green
	0.65	0.65	0.65	Olive green
	0.81	0.81	0.81	Olive green
	0.90	0.90	0.90	Light purple

n.d.: not detected

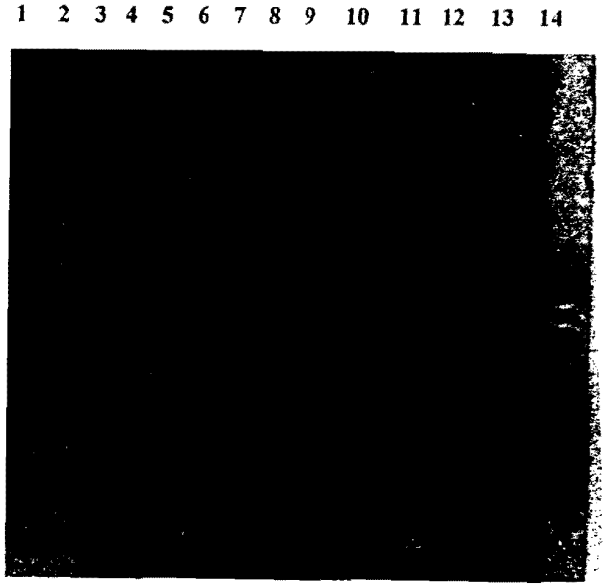
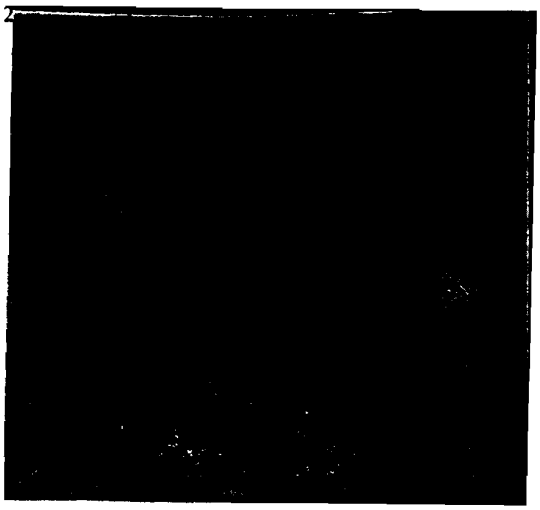


Fig (1): Chromatogram of ethanolic extract of microwaved pre-soaked soybean seeds compared with white saponin
Lanes 1, 8, and 12 (0. sec.), Lanes 2, 5, and 9 (160 sec.)
Lanes 3,6, and 10 (120 sec.), Lanes 4,7, and 11 (80 sec.)
Lane 13 (raw), Lane 14 (white saponin)
1 2 3 4 5 6 7 8 9 10 11



Soaking (12 hr.) (6 hr.) (1 hr.)

Fig (2): Chromatogram of ethanolic extract of microwaved pre-soaked kidney bean seeds, compared with white saponin.
Lanes 1, 4 and 7 (160 sec.), Lanes 2,5 and 8 (120 sec.)
Lanes 3,6 and 9 (80 sec.),
Lane 10 (raw), Lane 11 (white saponin) .

Saponins are steroid or triterpenoid glycosidic compounds and are not highly water soluble, so leaching of the saponins from the tissue would be minimal. Therefore, a possible mechanism for the observed decrease in the saponin content of the soaked-microwaved beans is the hydrolysis of the glycosidic bonds between the sapogenin and glycosidic residue during thermal processing.

On the other hand, Fenwick and Oakenfull (1983) concluded that thermal processing did not destroy saponins.

For irradiation treatment, TLC chromatograms (Figs: 3 and 4) showed no detectable differences between the TLC patterns of crude saponin of the non-irradiated and irradiated pre-soaked legume seed samples.

No significant variations in number and intensity of saponin spots of both legume seeds with varying irradiation doses up to 7.5 KGy. This is finding in agreement with Kwon *et al* (1990). They stated that ginseng saponins were found to be very stable to γ-irradiation. Also, Cho *et al* (1985) reported that irradiation doses up to 20 KGy caused negligible changes in HPLC and TLC patterns of crude saponins extracted from leaves and roots. It can be concluded that γ-irradiation treatment up to 7.5 KGy is not effective treatment for reducing saponin level in legumes in comparison with microwave treatment.

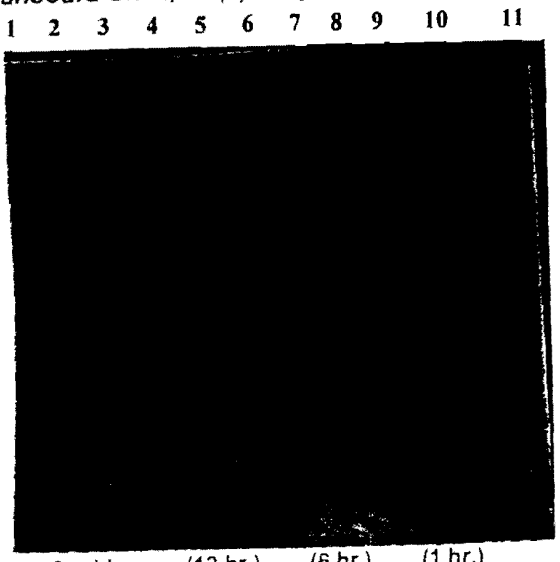


Fig (3): Chromatogram of ethanolic extract of soaked pre-irradiated soybean seeds compared with white saponin.
Lanes 1, 4 and 7 (7.5 Kgy), Lanes 2, 5 and 8 (5.5 Kgy)
Lanes 3, 6, and 9 (2.5 Kgy), Lane 10 (raw) , Lane 11 white saponin)

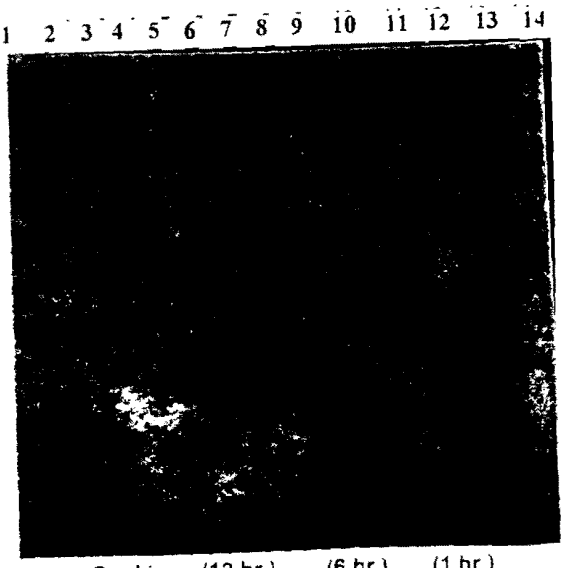


Fig (4): Chromatogram of ethanolic extract of soaked. pre-irradiated kidney bean seeds compared with white saponin.
Lanes: 1,5, and 9 (7.5 KGy), Lanes: 2,6 and 10 (5.5 KGy)
Lanes: 3,7 and 11 (2.5 KGy), Lanes: 4,8 and 12 (0 KGy)
Lanes: 13 (raw) and Lanes 14 (white saponin).

CONCLUSION

Fortunately most anti-nutritional proteins are heat-labile and on proper cooking conditions become non-inhibitory. Although most legumes are eaten cooked, it should be borne in mind that in developing countries of the third world the amount of fuel available for cooking is often limited and further more, since animals are fed raw seeds, large amounts of these antinutrients factors may influence animal nutrient utilization. On the other hand, cooking should not be too prolonged, as there may be a decrease in nutritional value. This decrease may be due either to destruction of vitamins, ascorbic acid and thiamin are particularly susceptible, for example to heat denaturation or modification of nutrients, in particular lysine and methionine. This is especially important in child nutrition since, unlike adults, children are often unable to renature partially denaturated nutrients (Boulter 1983).

Nevertheless, taking account of all these antinutrient factors, legumes still provide a valuable source of dietary protein.

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تأثير الميكروويف وأشعة جاما على بعض مضادات التغذيـــة فـــى بـــذور بعـــض البقوليات

ماجدة عويس محمـــود- محمدى عبد الحميد عيسى- حســـنى شفيق عبـــد الســـلام- محمد شعبان حسن- أمل أحمد صلاح

قسم الكيمياء الزراعية - كلية الزراعة - جامعة المنيا - مصر

يتناول هذا البحث:

- تأثير التسخين بالميكروويف على محتوى بذور صنفين جديدين من فول الصويا والفاصوليا هما Giza-35 و Paullista من مضادات التغذية .
- تأثير التشعيع بأشعة جاما على هذه المضادات .
- تقييم تأثير النقع أو التسخين سواءا باستخدام الميكروويف أو بأشعة جاما للبذور المنقوعـــة أو المنقوعـــة والتي سبق تشعيمها على خفض مستوى سكرات عائلة الرافينوز.
- كذلك تقييم هذه الأصناف الجديدة من حيث مكوناتها من مضادات التغذية .

وللوصول لذلك تم تشعيع بذور كل من فول الصويا صنف Giza-35 وبذور الفاصوليا صـــنف Paullista ثم نقع هذه البذور لمده ساعة ٦، ساعات ١٢، ساعة.وفي جزء أخر تم نقع هذه البـــذور للمـــدد السابقة ثم تسخينها بالميكروويف لمده ٨٠ ثانيه و ١٢٠ ثانيه و ١٦٠ ثانيه.أما الجزء الثالث من البذور فكان بدون معامله واستخدم كبذور خام (raw).

تأثير المعاملات على مضادات التغذية :-

١-سكرات عائلة الرافينوز:

كان السكروز في فول الصويا والفاصوليا (البذور الغير معاملة) ٣,٧٦،١,٩٤% على التوالي أما الرافينوز والاستاكيوز ٠,٧٥% و٠,٩٧% في بذور فول الصويا و٠,٩٥% و١,٦٥% في بذور الفاصـــوليا الغير معاملة .

- أدى التسخين لمده ٨٠ ثانيه بالميكروويف للبذور مسبوقة النقع (١٢ســـاعه) إلـــى نقـــص مســـتوى الرافينوز بمقدار ٩٤,٦٧% وسكر الاستاكيوز بمقدار ٩٢,٧٨% فـــي فـــول الصـــويا و ٩٤,٧٤% و٥٦,٣٦% في الفاصوليا على التوالي من محتواها الأصلي وادت نفس المعاملة إلى نقص السكروز بمقدار ٧٠,٢١% في فول الصويا و٥٨,٧٦% في الفاصوليا.

- تشعيع بذور فول الصويا والفاصوليا المنقوعة لمدة ٦ساعات أدى إلى نقص سكر الاستاكيوز حوالي ٥١,٨٦% ، ٣٥,٥٧% على جرعه ٢,٥ كيلو جراى و ٥٦,٩١%، ٣٨,٦٦% على جرعه ٥,٥ كيلو جراى عن الكميات الأصلية فى البذور الجافة على التوالي.

من ناحية أخرى لم يتمكن من كشف سكر الفرباسكوز فى البذور سواء المعاملة أو الغير معامله فى هذه الدراسة بواسطة التحليل الكروماتوجرافى بالطبقة الرقيقة TLC.

٢-حامض الفيتيك :

بإجراء التقدير الكمي لحامض الفيتيك تبين أن محتوى العينات (الخام) منزوعة الدهن كان ٣,٦٨ ملليجرام / جرام فول الصويا و ٣,٢٥ ملليجرام /جرام فاصوليا ووجد أن جميع المعاملات قد أدت إلى نقص محتواها من حامض الفيتيك وبزيادة مدة النقع مع طول مده التسخين أدى إلى نقص حمض الفيتيك. وقد سجل أقصى نقص فى محتوى حامض الفيتيك حوالي ٧٠% فى فول الصويا وحوالي ٦٠% فى الفاصوليا للبذور المنقوعة لمده ١٢ ساعة والمعاملة بالتسخين لمدة ١٦٠ ثانية بالميكروويف.كما أدى التشعيع على ٧،٥ كيلوجراى للبذور المنقوعة لمدة ١٢ ساعة إلى نقص فى مستوى حامض الفيتيك فى فول الصويا والفاصوليا حوالي ٥٣,٨٠% ، ٧٤,٧٧% على التوالي.

٣-نشاط مثبط إنزيم التربسين :

- زيادة مده التسخين بالميكروويف فى جميع المعاملات أدى إلى نقص نشاط مثبط إنزيم التربسين فبعد المعاملة بالتسخين لمده ١٦٠ ثانيه للبذور مسبوقة النقع لمده ١٢ ساعة انخفض نشاط مثبط إنزيم التربسين فى فول الصويا والفاصوليا بمقدار ٦١,٨٠% ، ٦٠,٢٥% على التوالي. بينما التسخين لمده ١٦٠ثانيه للبذور المسبوقة النقع لمده ساعة انخفض نشاط مثبط إنزيم التربسين بحوالي ٢٧,٨، ٢٩,٥% فى فول الصويا والفاصوليا على التوالي أما البذور مسبوقة النقع لمده ٦ساعات انخفض محتواها من مثبط إنزيم التربسين إلى ٥٥,٤ ، ٤١,٤٥%.
- البذور المنقوعة لمده ١٢ ساعة والتي قد تم تشعيعها على ٧,٥ كيلو جراى أنخفض محتواها من مثبط إنزيم التربسين إلى حوالي ٤٩,٨% ، ٦٠% على التوالي بالمقارنة بمستوياتها فى البذور الجافة

٤-الفينولات (التنينات):

لقد سجلت نتائج تسخين البذور بالميكروويف لمده ١٦٠ثانيه و المنقوعة لمده ٦ ساعات زيادة فى محتواها من الفينولات بمقدار ٢٧,٩١% ، ٣٣,٣٣% من الكميه الأصلية للبذور الجافة لفول الصويا والفاصوليا على التوالي.

- النقع لمده ١٢ ساعة ثم التسخين لمده ١٦٠ ثانيه أدى إلى زيادة كبيره للفينولات فى فول الصويا بحوالي ٣٤,٨٨ ، ٤٥,٣٣% فى الفاصوليا بينما التشعيع على ٧,٥ كيلو جراى للبذور المنقوعة لمده ١٢ ساعة أدى إلى زيادة محتواها من الفينولات بـ ١,٥،١,٦ مره فى فول الصويا والفاصوليا على التوالي.

٥-الصابونينات :

دلت النتائج على أن البذور الجافة لفول الصويا تحتوى على ٨ مكونات ألوانها تتراوح من الفوشيا (القرمزي) إلى الأخضر الزيتوني وبحساب قيم (R_F) لهذه المكونات ٠,٠٦، ٠,٢٥،٠,١٩،٠,٤٤ ومن ناحية أخرى فقد تبين أن المكونات ع ذات قيم R_f ٠,٠٥ ،٠,٦٥،متوسطة الكثافة والتي كانت قيمتها ٠,٨١، ٠,٩ كثافتها منخفضة أيضا فلقد لوحظ أن الصابونين الخام للبذور الجافة للفاصوليا قد أعطى أربع مكونات تتراوح قيمة(RF) ٠,٥، ٠,٦٥، ٠,٨١ ،٠,٩.بينما التحليل الكروماتوجرافى باستخدام الطبقة الرقيقة (T.L.C) لمستخلص الصابونين الخام للبذور المسخنة لمده ١٦٠ ثانيه مسبوقة النقع لمده ١٢ ساعة لفول الصويا والفاصوليا أعطت ٦،٢ مكونات على التوالي كما أظهرت عدم وجود اختلاف بين الصابونين الخام لبذور البقوليات مسبوقة النقع الغير مشععه والمشععه لجميع المعاملات .