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FUNCTIONAL PROPERTIES AND ANTINUTRITIONAL FACTOR OF SOME EGYPTIAN LEGUMES AS AFFECTED BY GAMMA IRRADIATION TREATMENT .

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ABSTRACT

The possibility of using Gamma irradiation to modify the functional properties and deactivate the trypsin inhibitor of the legumes and their soups were studied in five Egyptian legumes; Faba beans, Lentils, French beans, Cowpeas and Chickpeas. This effect was illustrated in two levels. The first level was at 1 KGy which is considered to be safe and appropriate dose for insect disinfestation; the other doses were applied at 5, 10 KGy.

There was a dose depended relationship between ($P<0.01$) the emulsification and oil absorption capacity and the absorbed dose. It seems that irradiation modify these properties. The data also showed a changes in the trypsin inhibitor destruction rate which was getting higher as a result of increasing the adsorbed doses. at (5, 10 KGy). Combining 1 KGy dose; the safe and appropriate for insect disinfestation with other traditional process led to complete deactivation of this inhibitor. The proposed mathematical model showed that Gamma irradiation affected the trypsin inhibitor through lowering the t1/2 half life and D value of the inhibitor.

Keywords: Egyptian legumes-Trypsin inhibitor - Gamma irradiation- Functional properties - Emulsification -Oil absorption

INTRODUCTION

Food irradiation is emerging as a major food processing and preservation technology in Africa; the initial scope of a co-ordinated research programme initiated by the Food and Agricultural organization / International atomic energy association (FAO/IAEA) is expanding from insect disinfestation of staple foods, to include traditional processed food products that could be safe and have extended shelf-life through processing involving irradiation (FAO/IAEA, 1997).

The Food and Drug Administration (FDA) had previously approved irradiation for several other products. The European Commission also recently achieved a general agreement on irradiation of food products (EC Directive food irradiation 1997). All these development may lead towards more use of irradiation processes for food preservation in the future. The major drawback of irradiation process is however consumer resistance. The use of irradiation technology is promising since its effects in nutrients is minimal if suitable doses are applied.

Among the components that are worthy to be emphasized on are trypsin inhibitors (TI) which are low molecular-weight seed proteins that are widely distributed among legumes. Many studies in monogastric animals have distributed major antinutritional effects. Principally a decreased growth

rate and pancreatic metabolic effects, due to the presence of TI in feed supplemented with legume seed meal. Protease inhibitors may inhibit growth, reduced digestibility and cause pancreatic hypertrophy (Linear and Kakade 1980).

Several conventional processing methods such as germination (Sathe et al 1983). Soaking (Hassen and El Tinamy 1995), and cooking (Vidal-Valuerde et al 1994) have been used to inactivate these undesirable components. The above mentioned treatments generally reduce raffinose oligosaccharides and antinutritional factors, but the effect varied with plant cultivars.

Irradiation had been suggested to remove or reduce the antinutritional factors (Ghazy et al 1992). Abou-Tarboush (1998) also found that a dose 10.0 KGy caused decrease in trypsin (by 34.9%) and chemotrypsin by (71.4%) whereas its invitro digestibility increased from 79.8 to 84.2%, So from this respect this study was carried out to investigate the possibility of using gamma irradiation to inactivate the antinutritional factors of five selected Egyptian legumes:- Faba beans, Lentils, French beans, Cowpeas and Chickpeas as well as their soup powders. In addition functional properties (Emulsification and oil absorption capacity) were also measured in relation to different irradiation doses. The applied doses were at:-

1- 1 KGy the safe and appropriate dose for insect disinfestations.

2- Other higher doses (5, 10 KGy) were also applied to determine the changes in the aforementioned properties of these legumes at various dose levels.

MATERIALS AND METHODS

1- Materials:

A full mature seeds of Faba beans *(Vicia faba)*; Lentils (*Lens esculenta*); French beans *(Phaseolus vulgaris)*; Cowpeas; *(Vigna spp)* and Chickpeas *(Cicer arietinum)* were obtained from the Agricultural Research Center; Horticulture Department. The seeds were kept under refrigeration (10^oC) prior to use.

2- Methods

2.1. Irradiation process:

The seeds were packed in individual polyethylene bags and irradiated at two different levels. The first level was at 1 KGy; the safe and appropriate for insect disinfestation; the second levels were at 5, 10 KGy doses. The process was carried out at 25^oC in a cobalt -60- Gamma irradiation unit of National Centre for Radiation Research and Technology (NCRRT). The dose rate was 0.6 $KGy.hr^{-1}$.

Sensory evaluation of the legumes and their soups irradiated with doses of 0, 1, 5, 10 KGy showed changes in the flavor (off flavor) at 10 KGy . that is why a dose of 10 KGy was excluded during the processing of the soups.

2.2.Soup powders preparation

legumes seeds were processed individually ; they were cooked in a small amount of water in a pressure cooker pan. They were processed until cooked soft. A lab tray cabinet drier was used in dehydration of all the cooked materials. Materials were spread on aluminum trays (40 ×60 cm) in a thin layer (about 3-4 mm thickness); put in an air ventilation oven at 60°-70°C for each individual legumes samples. Dehydration was continued until samples reached constant weight. The different dried samples were ground to a fine flour in a laboratory grinding machine; then sieved through a silk siever to about 95% extraction ratio by weight; tightly placed in polyethylene.

3- Measurement of the functional properties :-

2.3.1 Oil holding capacity was measured according to the method by Sosulski and McCurdy 1987. The oil holding capacity was expressed as a number of ml of oil held by 1g of flour.

2.3.2 Emulsifying activity was measured according to the method by Sathe et al 1983. Emulsifying activity (EA) was calculated as follows:-

EA%= Volume of emulsified layer x volume of whole layer in centrifuge tube $^{-1}$ x 100 .

2-4 Trypsin Inhibitor

2.4.1 Extraction of the seed proteins :-

Firstly, mature seeds were washed with water, dried and ground to make a fine powder. The fine powder was defatted by homogenization in 30 sec in 8 volumes of cold acetone; and the homogenate was filtered. The acetone in soluble materials were washed several times with acetone and finally once with ether and dried at room temperature overnight. The previous acetone extract was kept at -20°C for further application.

The acetone dry powder was extracted with 1:4 (w/v) distilled water for about an hour.

2.4.2 Precipitation with ammonium sulfate:

The extracts were combined and adjusted to pH 5.0-5.1 with 5M NH_4OH. The inhibitor was precipitated by adding solid ammonium sulfate with stirring to 50% saturation. After 2 hours; the precipitated protein was removed by centrifugation, the pH was adjusted. The extracts contained about 90% of the original activity.

2.4.3 Inhibitor assay:

Trypsin inhibitor assay was performed according to the method described by (Sarita et al 1996) using casein as a substrate.

Residual trypsin activity was estimated by measuring the absorption of supernatant at 280 nm. One trypsin unit is defined as the activity resulting in an increase of one unit of absorbance at 280 nm of TCA soluble casein hydrolysis product liberated by trypsin action at 37°C; The data were measured as a function of different incubation time to establish the stability criteria

2.5: Statistical analysis

The experiment was replicated three times. Experimental results were analyzed with the statistical analysis system (SAS Institute Inc 1985) by general linear model. Analysis included the use of variance to investigate the effect of different irradiation doses. In addition regression techniques (Drapper and Smith 1981) were also applied. Student t-test was also used to test the significance of the data at alpha level ($P<0.05$) and ($P<0.01$).

2.6: Model used to describe trypsin inhibitor inactivation:

The stability of trypsin inhibitor after irradiation was described using an exponential model by applying a semi-logarithm scale of the inhibitor residual activity vs reaction time (t); data were obtained from the results seen in fig (4).

Residual activity = $a.e^{kt}$……(1)

where

$Kmin^{-1}$: Reaction rate constant

Values for the inhibitor half.life (t ½) which is the time required for 50% reduction in initial activity was determined using.

$t ½ = Ln (0.5) k(min^{-1})$…….(2)

Values for (D) which is the duration of time after gamma irradiation treatment to reduce 10% of its original initial activity.

$D = 2.303 k(min^{-1})$…….(3)

The Z value which is the dose change (KGy) required to change the inactivation time by a factor of 10 and it was determined by the procedure described by (Singh and Heldman 1993).

RESULTS AND DISCUSSION

Measurements of the functional properties and trypsin inhibitor of irradiated legumes and their soups were carried out in this investigation at safe dose (1KGy) and also at higher dose (5 and10 KGy).

3.1: changes in the functional properties of irradiated legumes and their soups:-

Functional properties are one of the most important factors that determine the suitability of these legumes to be processed. In our study an attempt was carried out to modify the functional properties by gamma irradiation.

3.1.1 Irradiation at safe dose (1KGy):

Data in fig (1) and fig (2) showed a slight changes in the functional properties (Emulsification and oil absorption) as compared with the control samples. It seems that low dose irradiation at 1 KGy had a little influence on the functional properties of proteins under the studied legumes.

3.1.2 Irradiation at higher doses (5-10KGy):

Data in fig (1) illustrated the changes that occurred in the emulsification (EA%) and oil absorption (ml oil. g flour^{-1}); fig (2) as a function of different irradiation doses. It seems that these two parameters were positively dose depended relationship ($P<0.01$) in all the studied legumes and their soups.

Oil absorption capacity of a protein may depend on its capacity to entrap the oil **(**Kinsella 1976**).** The relatively high oil absorption capacity of irradiated legumes may be attributed to the degree of denaturation due to chemical modification. In the soup produced from irradiated legumes, an increment in oil absorption capacity was noticed. In general the amount of oil bound is markedly affected by the method used, the protein content, the surface area, the charge and topography, the hydrophobicity and the liquidity of the oil. It is conceivable that the binding capacity is enhanced by denaturation of the protein which exposes a polar amino acids**.**

Emulsification capacity (EA%) is enhanced by increasing different irradiation doses; fig (1) but during the processing of soups. Emulsification is reduced significantly ($P<0.05$). The legumes subunit has a limited effect on emulsification; but the unfolding and possibly aggregation that occur at high temperature reduce emulsifying activity. This effect may be due solely to decrease solubility. Excessive heating of legumes protein reduces emulsifying capacity (McWatters and Holmes, 1979).

Modification of legume protein improves their emulsifying properties. Thus gamma irradiation improves emulsifying capacity and enhances emulsion stability probably via electrostatic effects and enhanced hydration (Franzen and Kinsella, 1976).

Excessive hydrolysis which is induced by gamma irradiation may result in smaller peptides facilitates the formation of emulsion but these show decreased stability presumably because of limited cohesion between the proteins in the interfacial membranes (Beuchat et al 1977).

It has been demonstrated that gamma - irradiation caused molecular changes resulting in condensation or polymerization degradation; hydrogen-bond disruption and cleavage of inter molecular disulfide bonds (Casarett 1968).

Therefore it is assumed that such molecular rearrangement bring about changes in secondary structure and disruption of the native conformation of legumes proteins especially at higher dose level; so as previously mentioned by several authors; that 20 KGy irradiation induced partial denaturation and degradation, and secondary conformational changes in legume proteins; but irradiation doses up to 10 KGy, where the physical properties were improved (Byun et al 1993); caused negligible changes in legumes proteins. It was found by (Jun Kang and Woo Byun 1996) in their study on irradiated soybean that the microstructure of compressed cells and cotyledon epidermis was to be deformed by gamma - irradiation. Protein body structure was also deformed, becoming spike shaped with 20 KGy of irradiation.

Table (1) showed the correlation coefficient (R^2) between different irradiation doses and the changes in the functional properties of both

legumes and their soups. The data showed a significant positive correlation at ($P<0.05$) and at ($P<0.01$) in all the studied legumes; which indicated that significantly gamma irradiation had an effect towards the enhancement and modification of the functional properties of protein measured as emulsification % and oil absorption.

Table (1): Correlation coefficients, between both emulsification, oil absorption and changes in different irradiation doses (KGy*) at various legumes species.**

	Emulsification		Oil absorption	
	S.E	R^2	S.E	R^2
Faba beans	0.00291	0.999**	0.0411	0.932*
Faba beans soup	0.044	0.996**	0.0411	0.932*
Lentils	0.613	0.907*	0.033	0.975*
Lentils soup	0.529	0.934*	0.033	0.975*
French beans	0.00061	0.999**	0.065	0.953*
French beans soup	0.209	0.989**	0.130	0.931*
cowpeas	0.549	0.989**	0.0206	0.932*
Cowpeas soup	0.873	0.953*	0.0206	0.932*
Chickpeas	0.00057	0.999**	0.0233	0.969*
Chickpeas soup	0.0167	0.999**	0.0233	0.960*

S.E : Standard error of the samples *: Significance at 5% **: Significance at 1%
R^2: Correlation Coefficients * the doses were at 0,1,5, and 10.**

3.2.: Irradiation dependence of trypsin inhibitor inactivation

The radiostability of trypsin inhibitor in both legumes and their soups were measured as a function of reaction time. As previously mentioned by several authors that most of these legumes inhibitors showed considerable stability towards heat, pH and other factors. So, several attempts have been carried out to combine irradiation with different processes like cooking and autoclaving to get rid of the trypsin inhibitors. Fig (3) showed the result of gamma irradiation of legumes and their soups in destructing trypsin inhibitor; and fig(4) showed the changes in relative activities.

3.2.1 Irradiation at safe dose (1KGy).

The data in fig (3) showed the radiostability of these inhibitor as a function of different reaction time (min); In all the studied legumes; treatment with 1KGy dose to legume didn't cause noticeable change in the destruction of the inhibitor; It was destructed by (10-40%) in all the studied legumes after 300 min. It seems that the inhibitor is having a relatively high stability over the reaction period of time. In the prepared soups; the data showed relative instability of the inhibitor, could led to a destruction ratio more than 90%; as it seems combing Gamma irradiation dose (1KGy) with other process like, autoclaving, cooking and drying could inactivate the inhibitor. This result came with the conclusion described by (Kovas et al 1991) on his study on soybean that gamma irradiation all alone didn't cause any changes in the trypsin inhibitor, but dielectric heating did so.

3.2.2 Irradiation at higher dose (5KGy).

As in fig(3). Increasing the dose of gamma irradiation had also an effect on the trypsin inhibitor stability. Also the data showed a high correlation in all the studied legumes between the applied dose and the destruction ratio of the inhibitor.

Using Gamma irradiation itself to deactivate the trypsin inhibitor indicates the need for higher irradiation doses (Abou-Tarboush 1998). Sattar et al (1990) found a significant linear relationship between loss of trypsin inhibitor and germination time and the rate of trypsin inhibitor destruction with irradiation doses. As seen also from the same figure; that combining irradiation with other treatments (i.e processing of soup) could lead to complete destruction of the trypsin inhibitor.

Based on the algorithm seen in the material and methods that described the mechanism of trypsin inhibitor inactivation. The results were seen in table (2). Gamma irradiation influenced the trypsin inhibitor through lowering its t ½ and D values; also in the soup samples the inhibitor half life t½ and the D values are much lowered; as previously mentioned due to the processes that involved in the soup production.

Table (2): Efficiency of irradiation process for inactivation of trypsin inhibitors in different legumes varieties.

Legume Varieties	Dose (KGy)	K(min)$^{-1}$		t ½ (min)	D (min)	Z (KGy)
Faba beans	0	0.00306	(0.996)*	227	753	2.404
	1	0.00343	(0.995)*	202	671	(0.981)*
	5	0.022	(0.996)*	32	104	
Faba beans soups	0	0.0189	(0.998)*	37	122	7.44
	1	0.023	(0.992)*	30	100	(0.991)*
	5	0.0337	(0.998)*	21	61	
Lentils	0	0.00137	(0.999)*	505	1681	
	1	0.00156	(0.969)*	444	1476	1.845
	5	0.0018	(0.987)*	38.5	128	(0.979)*
Lentils soups	0	0.013	(0.997)*	53	177	14.08
	1	0.0143	(0.969)*	48	161	(0.996)*
	5	0.0188	(0.995)*	36	123	
French beans	0	0.00125	(0.999)*	555	1842	2.02
	1	0.00173	(0.989)*	401	1332	(0.995)*
	5	0.014	(0.998)*	50	165	
French beans soups	0	0.00513	(0.999)*	53	449	3.86
	1	0.00924	(0.998)*	48	249	(0.943)*
	5	0.0210	(0.996)*	24	110	
Cowpeas	0	0.00142	(0.997)*	488	1621	3.236
	1	0.00161	(0.997)*	430	1430	(0.987)*
	5	0.00625	(0.992)*	110	368	
Cowpeas soups	0	0.0144	(0.988)*	488	160	18.86
	1	0.0157	(0.939)*	430	147	(0.987)*
	5	0.0190	(0.976)*	110	121	
Chickpeas	0	0.00108	(0.998)*	642	2132	3.48
	1	0.00145	(0.999)*	478	1588	(0.99)*
	5	0.00456	(0.985)*	152	505	
Chickpeas soups	0	0.00412	(0.999)*	168	559	8.012
	1	0.00658	(0.970)*	105	350	(0.78)*
	5	0.00861	(0.988)*	81	267	

- **Values in brackets are correlation coefficient.**

CONCLUSION

From the previous results and discussion, it can be concluded that:

- Gamma irradiation induces changes in the conformational structure of proteins which lead to the enhancement of functional properties (Emulsification, and oil absorption) of the studied legumes. These enhancement might improve the cooking quality of the legumes.
- Combining gamma irradiation treatment at 1 KGy which is safe for insect disinfestation with other treatments (cooking; autoclaving; drying) proven to be successful in deactivating the trypsin inhibitor.
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الخصائص الوظيفية للبروتين والعوامل المعيقة للاستفادة من العناصر الغذائية فى بعض انواع البقوليات المصرية وتأثير المعاملة بأشعة جاما.

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تم فى هذا البحث التعرف على إمكانية استخدام أشعة جاما فى تعديل الخصائص الوظيفية للبروتين والتخلص من مثبط التربسين فى بعض انواع البقوليات المصرية وانواع الشوربة الناتجة منها. وقد أشتملت الدراسة على خمسة انواع من البقوليات المصرية وهى الفول – العدس –الفاصوليا – اللوبيا- الحمص. وقد تم دراسة هذا التأثير عند مستويين من الأشعاع المستوى الأول عند الجرعة 1 كيلوجراى وهى تعتبر جرعة آمنة ولها تأثير حافظ ضد مهاجمة الحشرات وقد أستخدمت كذلك جرعات عالية من 5و 10 كيلو جراى. كانت هناك علاقة وثيقة عند مستوى 0.01 بين الخصائص الوظيفية (درجة الاستحلاب- درجة امتصاص الزيت) ودرجة الجرعة الأشعاعية المستخدمة . وقد اوضحت الدراسة أن أشعة جاما قد عملت على التعديل فى هذه الخصائص السابقة. أيضاً قد بينت الدراسة حدوث تغير فى معدل تكسير مثبط التربسين بإزدياد جرعات الأشعاع (عند 5و 10 كيلوجراى). ولكن إضافة جرعة عند 1 كيلوجراى مع بعض المعاملات التقليدية قد ادت إلى تحطم كامل لهذا المثبط.
وبدراسة النموذج الرياضى المقترح قد تم إيضاح ان أشعة جاما تؤثر على مثبط التربسين عن طريق الإقلال من فترة نصف العمر وقيمة D لهذا المثبط.