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The Quality Evaluation of Cowpea Seeds as Affected by Gamma Irradiation 1- Evaluation of Cooking Aspects, Nutritional, Digestibility, Starch structure, Flatulent Effect and Sensory Improvement

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ABSTRACT

Cowpeas (*Vigna unguiculata* L. Walp) are leguminous seeds where they are important sources of proteins, vitamins, carbohydrates, fibers and minerals. However, some of their non-nutritive elements can present undesirable side effects like flatulence provoked by the anaerobic fermentation of oligosaccharides, such as raffinose and stachyose, in the gut. On the other hand, raw grains acceptability as staple foods is limited with the problem known as hard to cook phenomenon which normally occur during storage. A way to avoid these inconveniences, without any change in the nutritional value, is an irradiation process. The cowpea seed was irradiated with gamma radiation at dose levels of 2.5, 5 and 10 kGy. Radiation processing reduced the oligosaccharide content of cowpea and increased the in vitro protein digestibility was dose-dependent manner up to 10 kGy. Here, we evaluated the effects of gamma irradiation and/or cooking on the cooking properties in cowpea grains (*Vigna unguiculata* L. Walp) samples. Irradiation led to significant reduction in cooking time and cooking yield of cowpea. But, irradiation resulted in significant leaching of nutrients from cowpea seeds during cooking in a dose-dependent manner. At the same time there was no significant effect of the radiation on the sensory attributes like flavor, taste, texture and colour of the cowpea seeds. Similarly, the radiation did not affect significantly the acceptability of the treated cowpea seeds. Granule morphology under scanning electron microscopy revealed that granules were irregular or elliptical.

Key words: Cowpea seeds, Gamma irradiation, Cooking properties, Sensory evaluation, In vitro protein digestibility, Oligosaccharides.

Introduction

Legumes such as cowpeas (*Vigna unguiculata* L. Walp) are affordable sources of protein in diets of millions of people living in less developed countries of the globe, where incidentally protein-energy malnutrition is prevalent. In regions of chronic protein shortage, it provides food of fairly high nutritive value to both humans and domestic animals (Sosulski *et al*., 1987). Cowpea, which is regarded as a prominent food crop in the third world, has a great role to play in alleviating poverty and malnutrition in developing countries. As well, the ever escalating prices of animal protein sources and insufficient local production, has led to an increasing demand for cheap and good quality protein foods such as cowpea (Akinyele and Onigbinde, 1988).

Cowpea provide essential nutrients and high level of protein (about 25%) making it extremely valuable where many people cannot afford protein foods such as meat and fish (Akpapunam and Sefa-Dedeh, 1997) and are often referred to cowpea as poor man's meat, owing to their use as primary and cheap protein sources (Rivas-Vega *et al*., 2006). It is popularly known as lobia in Arabic world. It is one of the cheapest sources of plant protein widely used in the Egyptian diet. In many parts of the Arab world and North African countries including Egypt, cowpea seeds are consumed as

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cooked seeds with tomato, oil and spices alone or in combination with meat and eaten with bread or rice.

The economic uses of cowpea makes it a choice crop for serving food security needs of societies. They represent one of the dietary staples in many parts of the world (Odedeji and Oyeleke, 2011). Food irradiation is a physical process involving an energy-input that does not induce radioactivity in foods and sterilization that has the potential to protect such grains from insect's infestation and microbial contamination.

The cooking process is essential to prepare beans for consumption. Before cooking, the beans should be selected and kept submerged in water because the soaking and the cooking processes develop the sensorial characteristics of flavor and texture (De Olive *et al.*, 2001). Cooking, however, can destroy many of the essential nutrients in food. Therefore, combining cooking with irradiation could reduce the cooking time and contribute to the preservation of these thermo sensitive contents, since gamma irradiation has been recognized as a reliable and safe method for food preservation, improving the nutritional value and inactivation or removal of certain anti-nutritional factors in some proteinaceous leguminous seeds, thereby helps to provide food security (Tresina and Mohan, 2012).

Irradiation causes a reduction in cooking time of various legumes such as lentils (Celik *et al.*, 2004), mucuna seeds Bhat and Sridhar (2008); Bhat *et al.* (2008a), cowpea seeds (Abu and Minnaar, 2009) and soybean (Pednekar *et al.,* 2010). Further, Abu and Minnaar (2009) noted a significant leaching of nutrients from cowpea seeds during cooking, in a dose-dependent manner as was indicated by soluble solids. Several studies reported that irradiation doses not have a significant effect on the sensory evaluation for various pulses. Gamma irradiation of cowpea bean (*Vigna unguiculata L. Walp*) samples using doses of 0.0, 0.5, 1.0, 2.5, 5.0 and 10.0 kGy, result in softer grains than control grains, but 5.0 kGy is the ideal dose because it not alter the chemical and sensory characteristics of cowpea seeds (Lima *et al.,* 2011). As well, Ocloo *et al.* (2012) indicated that there was no significant effect of the radiation on the sensory attributes like flavor, taste, texture, softness and colour of cowpea.

Rehman and Shah (2001) observed an improvement in protein digestibility of black grams due to removal of tannins after pressure cooking. In vitro protein digestibility showed significant dose dependent increase (Tresina and Mohan, 2012). Similarity, Bamidele and Akanbi (2013; 2015) found a significant increase of in vitro protein digestibility in pigeon pea flour irradiated at 20 kGy. Irradiation apparently did not cause fissures or splitting in cowpea starch granules up to 50 kGy (Abu *et al.,* 2006).

Cowpea contains certain anti-nutritional constituents such as oligosaccharides (raffinose, stachyose and sucrose) and thought be the major cause of flatulence. Tresina and Mohan (2012) explored the Irradiation processing significantly reduces the levels of oligosaccharides of *Vigna unguiculata (L.) Walp subsp*. unguiculata when compared to the un-irradiated seeds. Also, cooking inactivates or reduces the levels of anti-nutrients such as flatulence-causing oligosaccharides, resulting in improved nutritional quality (Wang *et al*., 2008).

The objective of this study was to evaluate, the effects of combined gamma irradiation and cooking treatments on cowpea beans with respect to the following: (1) the cooking time; (2) the sensory attributes; (3) the In vitro protein digestibility; (4) morphological properties; (5) the oligosaccharide contents of cowpea (*Vigna unguiculata* L. Walp)

Materials and Methods

Materials:

The cowpea seeds (*Vigna unguiculata* L. Walp), locally type called Tiba were obtained from the Agricultural Research Center, Cairo, Egypt, in the season of 2014.

Methods:

Sample Preparation:

After the removal of foreign materials from cowpea seeds (such as broken, immature and damaged seeds**,** sands, stones and wastes), the cleaned cowpea seeds were divided into eight groups which differ in between irradiated, non-irradiated, cooked and uncooked, as clearly shown in Figure (1).

Processing of cowpea seeds:

Gamma Irradiation treatment:

The samples of cowpea seeds (250 g per each) were packaged in polyethylene bags, and sealed by heat and irradiated at ambient temperature by gamma irradiation from (^{60}Co) source operated in the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The facility used was the Indian Gamma Chamber 4000 A, ^{60}Co. The applied irradiation doses were 2.5, 5 and 10 kGy, delivered at a dose rate of 2.21 KGy/h as calibrated using small pieces of the radio chromic film at the time of experimentation (McLaughlin *et al*., 1985).

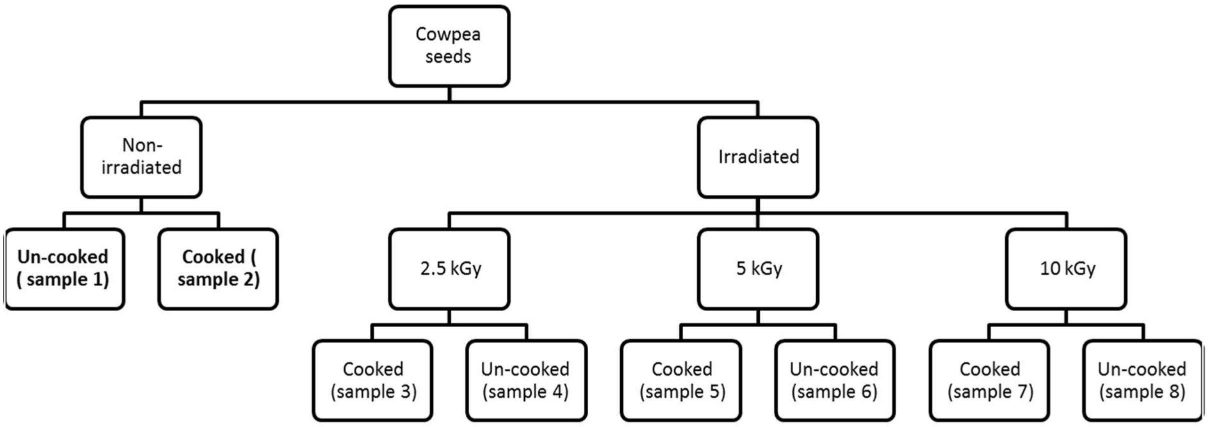

Fig. 1: Show the eight samples of cowpea seeds used in this work

Heat processing (Cooking):

The whole cowpea seeds (one of non-irradiated and three of irradiated samples at three tested levels) were cooked following the common method of home cooking used in Egypt, in which the seeds were cooked at normal fire (~ 100 ^{0}c) for the optimal cooking time. The un-cooked samples were also taken as control.

Analytical methods:

Cooking characteristics:

The methods of Wang *et al.* (2003) and Chinma *et al*. (2008) were used for determination of the cooking time, soluble solid losses in cooking water and cooking yield characteristics.

In-vitro protein digestibility:

The Bamidele and Akanbi (2015) method was employed to determine the in-vitro protein digestibility of cowpea flours, using pancreatic enzymes that have proteolytic activity.

Determination of anti-nutritional factors:

Oligosaccharides of cowpea flour were evaluated by using high-performance liquid chromatography (Agilent 1200, series) according to Zielinski *et al*. (2014). This analysis was performed at the central laboratories of the Agricultural Research Center, Egypt.

Scanning electron microscope (SEM):

The starch granules of raw and irradiated samples of cowpea seeds at 10 kGy were inspected by scanning electron microscope. The samples were dried in an air convection oven at 40 °C to a constant weight. The dried seed samples were separately milled /ground in a grinder and passed through a sieve of 150 meshes. A thin layer of each sample was placed on an adhesive tape attached to a circular aluminum specimen stub. The samples were coated by gold sputter coater (SPI-Module). Finally the samples was viewed under scanning electron microscopy (JEOL-JSM-5500LV) at 20 kV by using high vacuum mode and the micrographs were viewed, recorded and downloaded. This work was conducted at the Regional Center of Mycology and Biotechnology, Cairo, Egypt, using the methods of (Sofi *et al.,* 2013).

Sensory evaluation of cooked cowpea grains:

The cooked cowpeas were organoleptically evaluated for color, flavor, texture, taste and overall acceptability using twenty membered of Food Science and Technology department. They were independently evaluated using the numerical difference test technique as described by Larmond (1977).

Statistical Analysis:

Statistical analysis were performed using computer program Statistical Packages for Social Science (SPSS, 2012) and values were compared with each other using one-way analysis of variance (ANOVA) followed by the Duncan's post hoc multiple test at a 5% probability level (Duncan, 1955).

Results and Discussion

Effect of gamma irradiation on the cooking properties of cowpea grains:

Cowpeas, as the other legumes, have been used as a low cost source of protein; it also contain a considerable proportion of carbohydrates as a source of energy. However, their consumption is limited due to difficulty in cooking and flatulence caused by oligosaccharides (Thakur and Singh, 1994).

The cooking process (combination of heating and hydration) is essential to prepare cowpea for consumption. The duration time required to cook a bean is normally long and mainly dependent on the permeability of the seed coat and on the gelatinization of starch (Bourne, 1982). However, longer cooking time is associated with some negative effects such as reduction in nutritive value of proteins and cause high nutrient losses which limiting their preference as protein source (Chandrashaker *et al*., 1981),

Table (1) depicts the cooking time, solid losses in cooking water and cooking yield of non-irradiated and irradiated cowpea at 2.5, 5 and 10 kGy.

Table 1: Effect of gamma irradiation on the cooking properties of cowpea.

Irradiation dose	Cooking time (min)	Solids in cooking water (%)	Cooking yield (%)
Control	42 ^{a} ±1.12	4.9 ^{c} ±0.06	124 ^{a} ±0.058
2.5 kGy	41 ^{a} ±0.57	5.2 ^{c} ±0.11	119 ^{b} ±1.15
5 kGy	38 ^{b} ±1.12	5.6 ^{b} ±0.17	113 ^{c} ±0.58
10 kGy	35 ^{c} ±0.57	6 ^{a} ±0.06	106 ^{d} ±1.15

The obtained results are represented the mean of triplicate determination. Standard error; the means in the same column followed by the same letter are not significantly different from each other at p>0.05 according to Duncan multiple range test.

It could be observed that the optimal cooking time of cowpea was considerably reduced with increasing radiation dose up to 10 kGy. These findings are in close of that observed by Ashaye (2008); Bhat and Sridhar (2008) and Pednekar *et al.* (2010), since they reported that cooking time of irradiated grains was considerably depended on the irradiation dose, where at high level of irradiation the cooking time is considerably reduced.

Gamma irradiation of cowpea seeds led to significant reduction in the cooking time of cowpea seeds at all of the doses might have been partially due to a more porous seed structure because of irradiation-induced structural changes such as degradation of starch and pectic substances leading possibly facilitated heat and mass transfer across cowpea seed shell during cooking thereby enabling seeds to be soften more quickly responded to cooking heat at short time than non-irradiated samples, these results agreement with (Wu *et al.,* 2002 and Abu *et al.,* 2005). Also, ionizing irradiation is known to increases activity of polymer-water interaction of carbohydrate, pectic substances and denatured proteins, which reduce cooking time (Rao and Vakil, 1985). Thus, irradiated cowpeas seeds could be cooked in less time than that non- irradiated which enhance cooking quality were improved.

The solid loss in cooking water of cowpeas was increased with increasing radiation dose up to 10 kGy. It was found that, as in Table (1), the irradiation at 5 kGy showed the lowest solids loss while using of 10 kGy led to increase the loss of solids in cooking water. This may be related to the leaching of nutrients from irradiated cowpea seeds during cooking that increased by increasing the radiation dose, since this loss was also depended on the permeability of seed shell which more facilitated by irradiation doses. Abu and Minnaar (2009) also reported that increasing in the solids in cooking water of irradiated cowpea was attributed to variety of means induced molecular and structural changes that may, in turn, facilitate nutrient migration out of the seeds during cooking.

The cooking yield of cowpeas was decreased with increasing radiation dose up to 10 kGy. Gamma irradiation reduced cooking yield because it cause an increase in the solids present in cooking water.

Effect of gamma irradiation on the sensory evaluation of cowpea grains:

Sensory properties of cooked gamma irradiated and cooked cowpeas as measure to quality are presented in table (2). It could be observed that the sensory evaluations of irradiated samples were slightly higher, but not significantly ($p > 0.05$), than those of non-irradiated samples even though at 10 kGy.

The radiation doses used did not affect the cowpea seeds acceptability since; the scores for taste, flavor, color and texture of irradiated samples were higher, but not significantly than those of non-irradiated samples.

Irradiated cowpea at the higher dose (10 kGy) had an acceptance which was slightly higher than the control. Such irradiated cowpea was partially acceptable in texture and flavour, but fully acceptable in taste. As can be seen in the present results, irradiated cowpea grains were slightly softer than the control grains; this is clarified by (Lima *et al*., 2011) whose found that Gamma irradiation of cowpea bean (*Vigna unguiculata* L. Walp) samples using doses of 0.0, 0.5, 1.0, 2.5, 5.0 and 10.0 kGy, result in softer grains than control grains.

Table 2: Effect of gamma irradiation on the sensory evaluation of cowpea seeds.

Parameter / Treatment	Color	Taste	Texture	Flavour	Overall acceptability
Control	$8.12^{a} \pm 0.01$	$7.60^{b} \pm 0.05$	$7.32^{c} \pm 0.01$	$6.81^{a} \pm 0.02$	$8.66^{a} \pm 0.20$
2.5 kGy	$8.15^{a} \pm 0.02$	$7.68^{ab} \pm 0.01$	$7.50^{b} \pm 0.06$	$6.95^{a} \pm 0.03$	$8.75^{a} \pm 0.06$
5 kGy	$8.17^{a} \pm 0.04$	$7.75^{a} \pm 0.05$	$7.81^{a} \pm 0.02$	$6.99^{a} \pm 0.05$	$8.87^{a} \pm 0.02$
10 kGy	$8.14^{a} \pm 0.02$	$7.77^{a} \pm 0.01$	$7.43^{bc} \pm 0.03$	$6.91^{a} \pm 0.10$	$8.80^{a} \pm 0.06$

The obtained results are represented the mean of triplicate determination. Standard error; the means in the same column followed by the same letter are not significantly different from each other at p>0.05 according to Duncan multiple range test.

But, among the dose values used, it can be observed that 5.0 kGy is the ideal dose because it have a high rating score of sensory panelists. These results agree with those obtained by (Lima *et al.,* 2011) and (Marathe *et al.,* 2016) whose recorded slightly higher scores for 5 kGy treated beans which were better than 10 kGy treated beans.

Also, Chaturvedi *et al.* (2013) revealed that, sensorial properties of gamma irradiated products depend on quantity of dose given and the composition of the product. It can be concluded that gamma irradiation can improved the sensory evaluation of cooked cowpea seed. Although the sensory assessments were encouraging that a market survey is necessary to determine the market performance and acceptability of the product.

It can, therefore, be deduced that ionizing irradiation did not mar acceptability of cooked cowpeas and the sensory attributes which did not significantly influenced by the radiation doses used. Generally, these results are in agreement with those obtained by (Ashaye, 2008) and (Ocloo *et al*., 2012) whose indicated the sensory attributes for the cowpea for both irradiated and non-irradiated samples were not significantly influenced by the radiation doses used.

Effect of gamma irradiation and/or cooking on the oligosaccharides contents (g/100g) dry matter of cowpea grains:

Like other legumes flatulence is the common symptom associated with consumption of cowpeas and more serious accompanying consequences like abdominal pain and diarrhea (Salunkhe and Kadam, 1989). The oligosaccharides as raffinose and stachyose, common in legume seeds are indigestible by monogastric animals in the small intestine (Leske *et al*., 1993). It is well know that these saccharides are causative agents of flatulence in humans, due to the lack of α-1, 6-galactosidase in the intestinal mucosa, so that it pass into the colon where they may produce diarrhea, flatus gas (Co_2, H_2 and small amounts of CH_4 gases) by the anaerobic fermentation of oligosaccharides and their inevitable social discomfort (Vijayakumari *et al.,* 1996). Both of raffinose and stachyose comprised of one and two galactose units joined together with sucrose in α-D linkages, respectively. Any process to broken or reduce such oligosaccharides could be prevent the flatulence of cowpea seeds. It is found in this present study that use of irradiation with cowpea seeds, before cooking was led to a decreasing oligosaccharide content in irradiated and cooked cowpea seeds as shown in Table (3). The content of oligosaccharides in cooked cowpea seeds was lower than control especially for 10 kGy treatment which was have the lowest oligosaccharide content among all treatments. These results were similar to the values found by (Lima *et al*., 2011).

These results conclusively indicate that the degradation of oligosaccharides occurred in a dose dependent manner; the increase in irradiation doses was accompanied with a decrease in oligosaccharide in the cowpea seeds. Gamma irradiation may break glycosides linkages in oligosaccharides to decrease the content of oligosaccharides. Additionally, the reduction effects on these oligosaccharide contents were even larger for 5.0 and 10.0 kGy irradiated compared to the control sample. It could be concluded that the radiation process of cowpea seeds is a successful way to avoid this inconvenience, without any change in the nutritional value.

Table 3: Effect of gamma irradiation during storage on the oligosaccharides contents (g/100g) dry matter of cowpea seeds.

Saccharides / Treatment	Saccharides content as affected by			
	Non cooked		Cooked	
	Raffinose	Stachyose	Raffinose	Stachyose
Control	0.17	3.85	0.084	2.82
2.5 kGy	0.14	3.59	0.069	2.32
5 kGy	0.12	3.37	0.050	2.24
10 kGy	0.06	3.14	0.014	1.87

No statically was accomplished for previous results

Effect of gamma irradiation on physicochemical of cowpea seed starch:

The scanning electron micrographs (SEM) of the two cowpea samples (raw and irradiated at 10 kGy) are presented in Fig. (2), the starches of these samples were clearly visible under SEM at 2500 magnification.

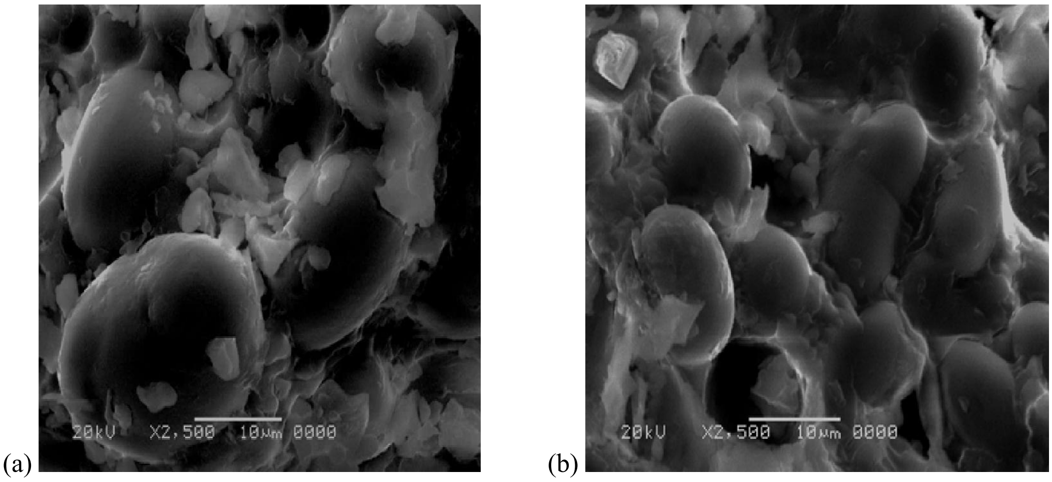

Fig. 2: Scanning electron micrographs (SEM) of control (a) and 10 kGy-irradiated (b) cowpea flours (2500 x mag.)

SEM results show that cowpea starch granules have relatively oval shape this agreement with that found by Abu *et al*. (2006) who reported cowpea starch granules are made up of several shapes (oval to kidney).

As a result of irradiation, the big granules of raw cowpea starch (Fig 2a) was degraded or splitted to less molecules granules (Fig 2b) and also formed more porous or vacuoles as also shown in (Fig 2b). Our results are similar to fairly with these reported by Chung and Liu (2009) whom exist that the irradiation damage the starch granules of maize and induced structure changes of starch molecules. Also, Abu and Minnaar (2009) reported that the reduction in cooking time of cowpea seeds might have been due to the pores induced in seed structure by irradiation at 10 kGy before cooking. Further, they added that irradiation process was degraded the starch granules and pectic substances. Incontrust, Agunbiade and longe (1999) and polesi *et al*. (2016) reported that the starch granules did not affected by irradiation.

Effect of gamma irradiation on in-vitro protein digestibility of cowpea grains:

It has been shown that the *in-vitro* protein digestibility (IVPD) can be comparable to *in vivo* protein digestibility in the rat model (Saunders et al., 1973). IVPD of control and irradiated cowpea flour are presented in Table (4).

The present study revealed that the IVPD of cowpea flour shows a dose dependent. Similarly, Increasing of IVPD after irradiation of cereals and legumes has been reported by Bamidele and Akanbi (2013; 2015)

Table 4: Effect of gamma irradiation on in-vitro protein digestibility (%) of cowpea grains:-

Treatment	% Protein digestibility as affected by	
Controls	Non-cooked	Cooked
	73.21	75.34
2.5 kGy	75.30	78.42
5 kGy	78.20	80.65
10 kGy	81.43	83.37

No statically was accomplished for previous results

The protein digestibility of irradiated and irradiated cooked cowpea seeds were increased as the irradiation dose increased. The combination of irradiation and cooking result in on increase in protein digestibility than that of non-cooked cowpea seeds. Similar results were obtained by Park *et al*., (2010) since they reported that cooking of irradiated pea seeds (*Pisum sativum* L) increased its protein digestibility.

Such increase has been attributed to the degradation of proteins into small fractions which be more susceptible to enzymes, Also, a partial destruction of trypsin inhibitors that occurred by irradiation was increased the IVPD as reported by Abu-Tarboush (1998); Rehman and Shah (2001); Bhat *et al*. (2008b) and Park *et al*., (2010).

Therefore, on the basis of these results, it is concluded that protein digestibility of irradiated cowpea seed was improved after cooking. It seems that the combination of cooking and irradiation was optimal for providing the much highest protein digestibility.

Conclusions:

Finally it could be concluded that irradiation treatment is a useful treatment to decrease cooking time, oligosaccharide content of cowpea seeds without any detrimental effect on the sensory characteristics which enhance the nutritional quality of cowpea seed. Especially when a combined with cooking process.

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