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Effect of radiation processing on antinutrients, in-vitro protein digestibility and protein efficiency ratio bioassay of legume seeds

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Abstract

The effects of irradiation (dose levels of 5, 7.5 and 10 kGy) on nutritive characteristics of peas (*Pisum satinum* L), cowpeas (*Vigna unguiculata* L.Walp), lentils (*Lens culinaris* Med), kidneybeans (*Phaseolus vulgaris* L), and chickpeas (*Cicer arietinum* L) were examined. Analyses included proximate composition, levels of anti-nutrients (phytic acid, tannins), available lysine (AL), in vitro protein digestibility (IVPD), and protein efficiency ratio (PER) in the growing rat. The results showed that moisture, crude protein, crude fat, crude fiber, and ash were unchanged by the irradiation. Radiation processing significantly ($p<0.05$) reduced the levels of phytic acid (PA), tannins (TN), and AL. IVPD and PER were significantly enhanced in a dose-dependent manner, relative to unirradiated control samples, for all legumes. The data sets for each legume exhibited high correlation coefficients between radiation dose and PA, TN, AL, IVPD, and PER. These results demonstrate the benefits of irradiation on the nutritional properties of these legumes.

Keywords: Legumes; Antinutrients; Phytic acid; Tannins; Radiation processing; Available lysine; Digestibility; PER; Rats

1. Introduction

Legumes are a cheap and valuable potential source of protein, and they are consumed in large quantities in Middle East countries. Legumes such as peas (*Pisum satinum* L), cowpeas (*Vigna unguiculata* L. Walp), lentils (*Lens culinaris* Med), kidneybeans (*Phaseolus vulgaris* L), and chickpeas (*Cicer arietinum* L) are consumed widely in Egypt. These legumes are valuable sources of complex carbohydrates, protein and dietary fiber, contribute significant amounts of vitamins and minerals, and high energy value (Morrow, 1991; Nielsen, 1991; Tharanathan and Mahadevamma, 2003). Protein contents in legume grains range from 17% to 40%, contrasting with 7–13% of cereals, and being equal to the protein contents of meats (18–25%) (de Almeida Costa et al., 2006). Poor nutritive values of the food legumes, due to the presence of some antinutritional substances, such as protease inhibitors, lectins, phytate, tannin and dietary fiber, including resistant starch, have been reported (Morrow, 1991; Siddhuraju et al., 2000, 2002). Tannins (TN) inhibit the digestibility of protein, whereas phytic acid (PA) reduces the bioavailability of some essential minerals (Van der Poel, 1990; Rehman and Shah, 2006). It has been observed by earlier workers, that radiation processing improves the nutritional quality of food legumes to various extents. Improvement in protein quality of soybeans and broad beans has been reported after the partial removal of trypsin inhibitor and haemaglutinin as a result of a radiation processing simple (Farag, 1998; El-Niely, 2001). PA, α-amylase inhibitor and oligosaccharids, were inactivated to a considerable extent when legume sample were irradiation Siddhuraju et al., 2000, 2002).

The literature has many reports demonstrating that thermal processing methods improve the nutritional quality of food legumes due to reduction in anti-nutrients. However, there is a scarcity of information relating to the effects of processing with ionizing energy. Therefore, the present work was undertaken to explore the effects of radiation processing on anti-nutrients and protein quality of food legumes.

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2. Materials and methods

2.1. Materials

Peas (*Pisum satinum* L), cowpeas, lentils (*Lens culinaris* Med), kidneybeans (*Phaseolus vulgaris* L), and chickpeas (*Cicer arietinum* L) were obtained from the Agriculture Research Institute, Ministry of Agriculture and Land Reclamation, Giza, Egypt. The samples were cleaned to remove broken seeds, dust and other foreign matter manually.

2.2. Radiation processing

About 5 kg lots of each sample were packed in well-sealed polyethylene sleeve. They were subjected at room temperature to gamma irradiation at dose levels of 5, 7.5 and 10 kGy, as monitored by FWT-60-00^{TM} radiochromic film. The radiochromic dosimeter was purchased from Far West Technology, Inc., Goleta, California, USA (ASTM, 2002 [ISO/ASTM 51275:2002(E)]).

The irradiation facility used was Egypt's Mega Gamma-1 Type J-65000 located at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The processed and raw samples were ground to pass a 20 mesh size screen, and stored in a freezer (−20 °C) until used.

2.3. Chemical analysis

Chemical composition (moisture, crude protein, crude fiber, ether extract and ash) of the raw and processed seed samples was determined by standard AOAC (1990) procedures. PA was extracted in 0.5 M nitric acid by shaking at room temperature for 3 h and assayed spectrophotometrically at 512 nm, as, described by Davies and Reid (1979). Tannin contents of the samples were estimated by spectrophotometer at 760 nm, using the Folin–Denis reagent after extraction with 1% hydrochloric acid in methanol (AOAC, 1990). The content of available lysine (AL) of samples was assayed according to Hurrell and Carpenter's method (1976).

2.4. In vitro protein digestibility (IVPD)

In vitro protein digestibility (IVPD) of all employed samples was determined by the extent to which the pH drops from pH 8 when the samples are subjected to sequential digestion with a multi-enzyme mixture using a modification of the multienzyme technique according to Hsu et al. (1977) and Satterlee et al. (1979). The enzymes used in the in-vitro protein digestion study were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. These were porcine intestinal peptidase; porcine pancreatic trypsin (type IX); bovine pancreatic chymotrypsin (type II), and peptidase (registry number; 9031-95-3; 9002-07-7; 9004-07-3; and 9031-96-3, respectively). IVPD of the sample was then calculated using the following equation: Digestibility $\% = 234.84–22.56X$, where X is the pH recorded after a total digestion period of 20 min. The multienzyme solution was freshly prepared before each series of tests. All analyses of each sample were done in triplicate.

2.5. Protein efficiency ratio (PER) bioassay

The standard method (AOAC, 1995) for assessment of the protein efficiency ratio (PER) was followed. Male Albino rats weighing 55 ± 5 g were obtained from the animal facility of Nuclear Research Center belonging to the Egyptian Atomic Energy Authority, Inshas, Egypt. They maintained in the animal facility of NCRRT and housed individually in polypropylene cages. The cages were kept in a care room at 22–25 °C and $60 \pm 5\%$ relative humidity, with a photoperiod of 12 h. The rats were fed a standard pelleted diet and water, ad libitum, for an acclimatization period of 4 days. They were then randomly distributed into the experimental groups. Rats fed with casein (10%) as a source of protein served as the common control group. There were 20 experimental diets, corresponding to the 5 legumes, each at 4 dose levels (0, 5, 7.5, and 10 kGy). Ground legumes were incorporated into the respective experimental diets as a substitute for a portion of the corn starch to make up 10% protein in the final diets (Table 1). PER was carried out for 28 days according to AOAC (1994). Food consumption and body weight of rats were assessed weekly and at 10-day intervals. The PER values were corrected by a factor of 2.5 times the value for casein standard (AOAC, 1995). Animal experimentation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1985).

3. Results and discussion

3.1. Proximate chemical analysis

The gross compositions (moisture, crude protein, crude fat, crude fiber and ash) of studied legumes, in their raw state, exhibited that moisture content was found to be 128.8, 84.6, 98.4, 117.0, and 114.0 g kg^{-1} for peas, cowpeas, lentil, kidneybeans and chickpeas respectively. Cowpeas contained the maximum amount of crude protein (266.7 g kg^{-1}) flowed in descending order by kidneybeas (255.2 g kg^{-1}), peas (236.0 g kg^{-1}), chickpeas (218.0 g kg^{-1}), and lentil (217.7 g kg^{-1}). The crud fat content was high in chickpeas (64.3 g kg^{-1}), followed by lentil (33.6 g kg^{-1}), peas (24.9 g kg^{-1}), cowpeas (24.5 g kg^{-1}) and kidneybeas (12.5 g kg^{-1}). Peas generally contains a high amount of crud fiber (64.4 g kg^{-1}) while lentil has a low amount of crude fiber (53.3 g kg^{-1}). Among studied legume seeds the content of ash was present in kidneybeans (43.3 g kg^{-1}). The gross compositions of studied legumes, in their raw stat, were in agreement with data presented by other