Investigation of gamma irradiation and storage period effects on the nutritional and sensory quality of chickpeas, kidney beans and green lentils

Ayca AYLANGE*, Erhan IC, Berna OZYARDIMCI

Turkish Atomic Energy Authority, Saraykoy Nuclear Research and Training Center, Kazan, Ankara, Turkey

Abstract

The objectives of this study were to determine the effects of gamma irradiation and storage period on the content of the total carotenoids, the oligosaccharides raffinose and stachyose, and the vitamins thiamine (B1) and riboflavin (B2) in pulses. Chickpea, kidney bean and green lentil samples were subjected to gamma irradiation doses of 0.25, 0.50 and 1.0 kGy followed by storage at room temperature for 12 months. The total carotenoids content was measured by spectrophotometer. Raffinose and stachyose were determined by high-performance liquid chromatography (HPLC) with refraction index detection and thiamine and riboflavin concentrations by HPLC with fluorescence detection. The impact of the irradiation dose can be seen in the result of the total carotene tests for lentils. The three different irradiation doses applied did not have significant effects on the levels of riboflavin and thiamine. The effect of the storage period was found to be significant on the raffinose and stachyose content but there were no significant changes following the applied irradiation doses. In the sensory evaluation the testers were not able to differentiate between the 0.25, 0.50 and 1 kGy applied irradiation doses and the unirradiated samples. The results of these studies suggest that irradiation with 1.0 kGy gamma rays cause tolerable losses in the nutrients studied in chickpeas, kidney beans and green lentils.

Key words: Pulses, Gamma Irradiation, Nutritional quality, Sensory analysis, Storage

1. Introduction

* Corresponding author. Tel: +90 312 81017 36; fax: +90 312 815 43 07.
E-mail address: ayca.aylangan@taek.gov.tr; ayca.yarali@yahoo.com (A. Aylangan)
Pulses are a cheap and important source of plant-based proteins and are consumed in large quantities by the population in Turkey. They are valuable sources of complex carbohydrates, protein and dietary fiber, contribute significant amounts of vitamins and minerals, and have high energy value (El-Niely, 2007). Of the total sown area in Turkey, 806,000 hectares is utilized for pulses. Turkey produced 1,140 tons of pulses in 2013. The main pulses produced in Turkey are chickpeas (506,000 tons), kidney beans (195,000 tons) and lentils (417,000 tons). The amount of pulse consumption per capita is relatively large quantity compared with the world pulses consumption; for example chickpeas are 4.61 kg per year, lentils are 5.22 kg per year and kidney beans are 2.88 kg per year (Anonymous, 2013).

Generally, post-harvest losses in pulses (20 – 25 %) arise mostly from transportation and storage food pests. Over the centuries, efforts have been made to control storage losses and maintain the quality of foods. One of the main problems in domestic and export market is infestation by stored-product insects. Vast quantities of stored grains and pulses are lost annually as a result of insect infestation (Khattak & Klopfenstein, 1989).

The increasing volumes of commodities being traded worldwide has created an urgent need for effective disinestation treatments to prevent the dissemination of alien invasive pests (Cannon et al., 2012). Post-harvest control of insects in pulses is essential under quarantine regulations in many countries (El-Naggar & Mikhaiel, 2011). Current available methods for post-harvest control are based on fumigation (Jemâa et al., 2012). The traditional treatment is chemical fumigation due to its low cost, fast speed in processing and ease of use (El-Naggar & Mikhaiel, 2011). However, the banning of the fumigant methyl bromide for all purposes (including phytosanitary and preshipment uses) in the European Union has further increased the need for effective alternatives (Cannon et al., 2012). For insect disinestation in pulses and grains, irradiation may offer an attractive alternative to chemicals (Villavicencio et al., 2000).

Protecting food from insect infestation is one of the most important goals of irradiation and the process effectively eradicates insect pests at all life stages (Machaiah & Pednekar, 2002). Food irradiation in Turkey has a very promising future having contributed to the conservation of food, the reduction of post-harvesting losses, and the possibility of improving food availability. The U.S. Food and Drug Administration (FDA) has approved irradiation of
pulses up to 1.0 kGy. The irradiation process applied to pulses has already been described in the literature (Al-Kaisey et al., 2003; El-Niely 2007; Khattak & Klopfenstein, 1989; Lima et al., 2011; Villavicencio et al., 2000). However, no study has directly examined the effects of the gamma irradiation and storage period on the total carotenoids content, oligosaccharides (raffinose and stachyose) and B vitamins (thiamine and riboflavin) in chickpea, kidney bean and green lentil samples. Carotenoids are a class of natural fat-soluble pigments found in pulses. These pigments also play roles in human health, preventing certain types of cancer, cardiovascular diseases and eye disorders (Nunes et al., 2013). Thiamine (vitamin B1) and riboflavin (vitamin B2) are important essential vitamins in pulse products. Research has shown that the change in nutritional value caused by irradiation depends on a number of factors. Among these are radiation dose, type of food, packaging and processing conditions such as temperature and oxygen exposure during irradiation and storage time (Crawford & Ruff, 1996). For example, Fox et al. (1989) reported that some vitamins, especially B1, are partially lost during irradiation; however this loss can be minimized by choosing appropriate conditions. Research has also shown that the loss of riboflavin (vitamin B2) and thiamine is no greater that that experienced during thermal processing (Steele & Engel, 1992). Raffinose and stachyose have been implicated as the causative factor for flatulence and abdominal discomfort (Dixit et al., 2011). In the literature, it has been reported that various processing methods, including gamma irradiation, could be possible alternative and additional processing techniques for reducing antinutrients like raffinose and stachyose. The irradiation methods applied to some pulses have already been described in the literature and represent alternative ways to fight harvesting losses because they contribute to the disinestation of wheats, some type of pea and beans, grains and some pulses which allows them to keep their chemical and nutritional qualities and increases their shelf life (Khattak and Klopfenstein, 1989; Villavicencio et al., 2000; El-Niely, 2007; Hajare et al., 2007; El-Naggar and Mikhaiel, 2011; Lima et al., 2011). Studies pertaining to the effect of gamma irradiation on nutritional components and storage period of chickpeas, kidney beans and green lentils are limited. In the present work, gamma irradiation was applied to chickpeas, kidney beans and green lentils, at low doses (0.25, 0.50 and 1.0 kGy), and the effects on nutritional and sensory parameters
were evaluated during one year of storage (immediately after the irradiation process, 6 and 12 months).

2. Material and methods

2.1. Samples and chemicals

The nonfumigated chickpea and kidneybean samples were obtained from a company (Memişler Group, Mersin, Turkey), the nonfumigated green lentil samples were obtained from the Ministry of Food Agriculture and Livestock, Central Research Institute for Field Crops, Ankara, Turkey. The samples were cleaned, placed in polyethylene bags (500 g each) and irradiated at the doses of 0.25 kGy, 0.50 kGy and 1.0 kGy (target doses) from the $^{60}$Co source at Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Ankara, Turkey. Unirradiated samples served as controls. The absorbed dose was checked using the Harwell perspex dosimetry (Harwell Gammachrome YR®, Perspex Dosimeter, Batch 62, Harwell, UK). The amounts of the measured doses are given in Table 1. Raffinose, stachyose, riboflavin, thiamine, acid phosphatase, papain, alpha-amylase, beta-glucosidase, glyoxylic acid and glutathione were purchased from Sigma Chemical Co. The Millex membranes (0.45 µm) were obtained from Millipore and the Sep-Pak C18 cartridges were purchased from Waters Corporation (Milford Massachusetts, USA). All other chemicals were analytical grade.

Table 1 insert here

2.2. Measurement of total carotenoids

The total carotenoids were measured according to the criteria by Alasalvar et al. (2005). The homogenate was filtered through a Whatman No.4 filter paper and washed until the residue was colorless. Finally, extraction solvent was added to the filtrate to a total of 100 ml and the absorbances at 471 and 477 nm were measured against an acetone blank using a Jenway 6505 UV/Vis spectrophotometer. The total carotenoids were calculated according to the following equation.

$$\text{Total carotenoids (}) = (\text{Abs}_{\text{max}}/250) \times [(25\text{ml acetone} \times \text{dilution} \times 100) / \text{sample weight}]$$
2.3. **Analysis of oligosaccharides**

2.3.1. **Extraction of oligosaccharides**

The extraction of the raffinose and stachyose was conducted according to the process given by Xiaoli et al. (2008). The samples (1.0 g) were extracted 3 times with 10 mL 50% ethanol-water at a ratio of 10:1 (solvent to samples) in a water-bath at 50 °C for 30 min. After each extraction, the samples were centrifuged at 2500 g for 20 min. Supernatants from the three cycles of extraction were combined and concentrated using a rotary evaporator (Büchi, Switzerland), and then dissolved into 5.0 mL of the mobile phase of HPLC (acetonitrile-water 75:25, v/v, HPLC grade of acetonitrile). Before injection, all the samples were filtered through a 0.45 µm Millipore membrane.

2.3.2. **HPLC analysis of oligosaccharides**

The separations of the raffinose and stachyose from pulses were carried out using a Waters 2695 HPLC with a refractive index detector. Both the oligosaccharide and standard solutions were separated with a Separon SGX NH2 column 5 µM (250 mm x 4.0 mm), using acetonitrile-water (75:25, v/v) as mobile phase at a flow rate of 1.0 mL/min. The injection volume was 20 µL. The raffinose and stachyose were identified by comparing the retention times with those of standard sugars. The standards were prepared according to the specification given by Hou et al. (2009) at different concentrations in order to create calibration curves. The raffinose and stachyose concentrations were expressed in mg/100g.

2.4. **Analysis of B vitamins**

2.4.1. **Enzymatic extraction of B vitamins**

The extraction of riboflavin and thiamine was performed according to the method proposed by Batifoulier et al. (2006). Samples (2.5 g) were weighed in a 50 mL centrifuge tube and 23.5 mL of a 0.05 M sodium acetate solution (pH 4.5) was added and vortexed in a Heidolph Reax Top (LabPlant, England). Then, the tube was placed in a shaking water bath at 100 °C for 10 min. After cooling, 500 µL of acid phosphatase (20 mg mL⁻¹), papain (100 mg mL⁻¹), alpha-amylase (10 mg mL⁻¹) and beta-glucosidase (20 mg mL⁻¹), 1.25 mL of 1 M glyoxylic acid (1 M), 200 µL of 2% ferrous sulphate solution and 250 µL of 1% glutathione were
added. The solution was incubated for 18 h in a shaking water bath at 37 °C, then diluted to 50 mL with distilled water, vortexed and centrifuged at 5000 g for 10 min.

For the riboflavin determination, the supernatant was filtered through a 0.45 µm Millex filter and the filtrate was used for chromatographic analysis.

For thiamine, a 4 mL aliquat of the supernatant was added to 3 mL of 1% potassium ferricyanide in 15% sodium hydroxide, mixed by vortexing for 10 s and left to stand for exactly 1 min. This solution (10 mL) was passed through a SepPack C18 cartridge after it was conditioned with 5 mL of distilled water and 2 mL of methanol. Then the SepPack cartridge was washed with 10 mL of 0.05 M sodium acetate (pH 6.0) and eluted with methanol-water (70:30, v:v). The eluate was made up to 10 mL with methanol-water (70:30, v:v) in a volumetric flask and filtered through a 0.45 µm Millex filter. The filtrate was used for the chromatographic analysis of thiamine (as thiochrome).

2.4.2. High-Performance Liquid Chromatography (HPLC) analysis of B vitamins

For the thiamine and riboflavin determination, the HPLC separation was accomplished with a µ-Bondapak C18 column (150 mm x 3.9 mm, Waters Corporation, Milford, MA, USA) with Waters 2695 fluorescence detector (Waters Corporation, Milford, MA, USA). The mobile phase (isocratic conditions) consisted of 0.05 M sodium acetate-methanol (30:70, v:v, pH 6.0). The separation was performed at 30 °C at a flow rate of 1 mL min⁻¹. The fluorimetric detector operated at an excitation wavelength of 365 nm and an emission wavelength of 435 nm for thiochrome, and at an excitation wavelength of 422 nm and an emission wavelength of 522 nm for riboflavin. The injection volume was 20 µL.

2.4.3. Standard solutions for external calibration

Stock standards of thiamine (1 mg mL⁻¹) were prepared in distilled water. Four hundred milliliters of acetic acid (0.02 M) were added to 50 mg of riboflavin (0.1 mg mL⁻¹). Complete dissolution was obtained by moderate heating and shaking for 1 h. After cooling, the solution was adjusted to pH 4.5 with 2.5 M sodium acetate and made up to 500 mL with distilled water.

2.5. Sensory evaluation
The sensory evaluation of unirradiated and irradiated chickpeas, kidney beans and green lentils samples was carried out soon after treatment and after 6 and 12 months (Meilgaard et al., 1999). It was performed in order to evaluate the effect of irradiation processing and storage time on the quality parameters of these pulses. Control and irradiated samples were boiled according to normal cooking times, then served on white, paperboard plates. Each sample plate was identified by a three-digit random number. Eight trained judges (4 women and 4 men) were selected from Sarayköy Nuclear Research and Training Center. The judges were asked to evaluate the samples for their color, odor, flavor, undesirable flavor, chewiness and overall acceptability using the 9-point hedonic scale (9=like extremely, 7=like moderately, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 1=dislike extremely).

2.6. Statistical analysis

The data was analyzed using analysis of variance (ANOVA) followed by Duncan’s multiple range test using the IBM SPPS software version 21.00. The effects of dose and storage time were considered significantly different when \( p<0.05 \).

3. Results and discussion

3.1. Total carotenoids content

Table 2 shows the effect of irradiation and storage time on the total carotenoids content of the chickpea, kidney bean and green lentil samples. The total carotenoids in the chickpea control samples were within a very narrow range, 2.69 – 3.57 mg/100 g. For the irradiated chickpea samples, the total carotenoids ranged from 3.33 – 3.71 mg/100 g, 3.12 – 3.52 mg/100 g and 3.09 - 3.87 mg/100 g, in the 0.25, 0.50 and 1.0 kGy samples, respectively. Thus, the profile of total carotenoids in chickpeas was very stable and showed no significant change \( (p>0.05) \) after irradiation. The storage period also did not significantly alter the total carotenoids content in both the control and irradiated chickpea samples. We found similar results for the kidney bean samples. There was no significant effect \( (p>0.05) \) of irradiation, or storage time on the total carotenoids contents of the kidney bean samples. The total carotenoids in the green lentil control samples varied from 2.53 to 3.36 mg/100 g and results were 2.27 – 3.17 mg/100 g, 3.71 – 3.96 mg/100 g and 3.55 – 3.92 mg/100 g in 0.25, 0.50 and 1.0 kGy irradiated samples, respectively. Thus, the profile of total carotenoids in the green lentil samples showed significant change \( (p<0.05) \) after irradiation. The storage period did
not significantly \( (p>0.05) \) alter the total carotenoids content in both the control and irradiated green lentil samples. The impact of the irradiation dose can be seen in the result of the total carotene tests for green lentil samples, the storage time had no effect. The storage time and irradiation dose does not significantly affect chickpea and kidney bean samples in terms of the results of the total carotenoids content. Hajare et al. (2006 and 2007) also observed a similar profile in the carotenoids in some legumes and vegetables that were irradiated at a dose of 2 kGy.

Table 2 insert here

3.2. Oligosaccarides content

Oligosaccharides (raffinose and stachyose) were identified according to the retention time of the standard sugars. Table 3a and Table 3b summarizes the effect of irradiation and storage time on the raffinose and stachyose content of the chickpea, kidney bean and green lentil samples, respectively. The dose of irradiation applied had no significant \( (p>0.05) \) effect on the oligosaccharides content of the chickpeas and kidney beans, although the storage time did have a significant effect \( (p<0.05) \) (Table 3). In the green lentil samples, the level of the irradiation dose significantly \( (p<0.05) \) affected the oligosaccharides content. However, there was no effect after storage for 6 or 12 months.

Table 3a insert here
Table 3b insert here

In all samples used in this study, the raffinose and stachyose content did not decrease with irradiation dose increases. However, Machaiah & Pednekar (2002) described the effects of low dose γ-radiation (0.25 and 0.75 kGy) processing for insect disinfestation on functionally important sugars in the commonly used pulses, mung, Bengal gram, horse beans (val), horsegram, cowpeas and rajma. In these species, they reported that the degradation of the oligosaccharides increased as the irradiation dose was raised. Al-Kaisey et al. (2003) reported that the raffinose and stachyose content decreased as the radiation dose increased in broad bean samples irradiated at 0, 2.5, 5, 7.5 and 10 kGy. During irradiation there was a
rapid decrease of the raffinose family oligosaccharides. Complete depletion or elimination of the raffinose family oligosaccharides was achieved at 10 kGy of radiation dose (Al-Kaisey et al., 2003). They concluded that gamma radiation seemed to be an acceptable procedure to improve the quality of broad bean from the nutritional point of view. Dixit et al. (2011) found that the gamma irradiation of soybean seeds at different doses (0.5, 2.0 and 5.0 kGy) resulted in a dose dependent decrease in the raffinose family oligosaccharides. They reported that gamma irradiation at higher doses may break the glycosidic linkages in oligosaccharides producing more sucrose and decreasing the content of flatulence causing oligosaccharides. Also, Lima et al. (2011) reported that the reduction effect of the oligosaccharide content was more intense for raffinose and stachyose at doses of 5.0 and 10.0 kGy for cowpea beans.

3.3. Vitamin B content

Table 4a and Table 4b shows the thiamine and riboflavin content of the chickpea, kidney bean and green lentil samples, respectively. The results indicate non-significant losses in thiamine and riboflavin concentrations for the analyzed samples at three different irradiation dose (0.25, 0.50 and 1.0 kGy). In contrast, the storage time significantly affected ($p<0.05$) the thiamine and riboflavin concentrations of the samples. As seen in Table 4a, storage time affected the thiamine contents in the chickpea samples. A decrease in the concentration of thiamine was found when the storage time was longer (Table 4a). The thiamine concentration in the control and 1.0 kGy irradiated sample was 0.58 ppm at the start of the experiment (month 0). When compared to the results of the tests after 12 months of storage, the thiamine content in the control and 1.0 kGy irradiated sample were seen to have decreased to 0.31 and 0.37 ppm, respectively. The riboflavin content of the chickpea, kidney bean and green lentil samples remained within a very narrow range; 0.18 – 0.20, 0.09 – 0.17 and 0.17 – 0.23 ppm, respectively throughout the storage time. The vitamin B contents were affected by the gamma irradiation treatment at a dose of 1.0 kGy ($p<0.05$). However, these chemical properties were affected by the storage time in the chickpea, kidney bean and green lentil samples.

Table 4a insert here
Thiamine (B1) and riboflavin (B2) are two of the essential vitamins. They are sensitive to the irradiation process but their degradative loss is similar to losses experienced during heating or other food processes (Crawford & Ruff, 1996). At doses at and below 1.0 kGy applied in chickpea, kidney bean and green lentil, the changes in the thiamine and riboflavin content were not significant. Our results are in accordance with other studies on thiamine content of irradiated food at a dose level of about 1.0 kGy (Villavicencio et al., 2000). For the riboflavin content, Khattak & Klopfenstein (1989) reported that no losses of riboflavin had been found in maize, wheat and beans after radiation processing in the dose range of 0.5 to 5.0 kGy. Lima et al. (2011) reported that thiamine levels analyzed by HPLC showed no significant differences (using a 5% range) between the control samples and the different irradiation doses (0.5, 1.0, 2.5, 5.0 and 10.0 kGy) for cowpea bean grains.

3.4. Sensory evaluation of pulses

The sensory evaluation of both unirradiated and irradiated (0.25; 0.50 and 1.0 kGy) pulse samples were undertaken initially then after 6 and 12 months of storage at room temperature. Figures 1, 2 and 3 depict the sensory evaluation score of the control and irradiated chickpea, kidney bean and green lentil samples, respectively. The applied irradiation dose significantly affected ($p<0.05$) the chewiness, color and aroma of the irradiated chickpea samples when compared to the control samples, although storage time irradiation dose had no significant effect ($p>0.05$). There were no significant changes ($p>0.05$) in the sensory attributes of the kidney bean and green lentil samples during the storage period in applied irradiation dose. The members of the tasting panel were unable to distinguish irradiated and control samples in terms of taste. Thus, radiation doses of 0.25, 0.50 and 1.0 kGy did not affect the overall acceptability of the pulses (chickpeas, kidney beans and green lentils). The panelists were not able to distinguish between non irradiated samples and those that had received up to 1 kGy.

We have shown that a gamma irradiation treatment can be used as a phytosanitary measure without any significant effects on the pulse sensory quality. Ocloo et al. (2012) reported
similar observations in which they showed that gamma irradiation up to 1.5 kGy did not affect the general acceptability of cowpea seeds. Machaiah and Pednekar (2002) also reported no significant changes in the sensory quality of pulses that had received 0.25 and 0.75 kGy irradiation doses. Villavicencio et al. (2000) reported that two varieties of Brazilian beans showed no significant changes in sensory quality up to after doses of up to 1 kGy; the level permitted for insect disinfestation. However, at higher doses, the sensory quality was reduced. The changes of sensory properties such as color, odor and taste, increase as a function of the radiation dose was increased.

4. Conclusions

This study investigated the effects of low-dose gamma irradiation treatment (0.25, 0.50 and 1.0 kGy) and storage time (0, 6 and 12 months) on chickpeas, kidney beans and green lentils in terms of the total carotenoids, oligosaccharides, vitamin B content, sensory properties. The results showed that the irradiation treatment used for phytosanitary purposes did not have any adverse effect on the nutritional and sensory quality of chickpeas, kidney beans and green lentils. Irradiation appears to be superior to other alternatives when considered on the basis of quality maintenance. In view of these results, it is suggested that pulses should be subjected to irradiation processing as a phytosanitary method to prevent the introduction or spread of regulated pests without any important changes in nutritional and sensory parameters. International trade in produce that has been irradiated for phytosanitary purposes is growing rapidly. This study may contribute useful information regarding the sterilization of stored pulses using gamma irradiation, with proper consideration of nutritional and sensory properties. Packaging material and storage conditions (relative humidity, temperature) are important parameters with respect to preventing re-infestation of pulses. Otherwise, further study will be needed on gamma irradiation effects on the foaming and emulsion capacity, resolution, textural and viscosimetric properties of chickpea flour, ground lentil and their
protein isolates when stored for a long time and used as food ingredients in the prepared food industry.

Acknowledgements

The authors wish to express their deep appreciation to the Turkish Atomic Energy Authority (TAEK-A3.H1.P1.01) for financial support, The Mersin Memişler Group, Ministry of Food Agriculture and Livestock, and the Central Research Institute for Field Crops for material support. The authors also thank Dr. Suresh D. Pillai for their valuable comments. We are grateful to Mr. Zati Unal, Mr. Mehmet Dogan Tarakli and Mr. Talat Aydin for the dosimetry measurements and their assistance in the irradiation processes undertaken for this research.
References


Table captions

Table 1. Harwell perspex dosimetry results for target and measured dose (kGy) uniformity of chickpea, kidney bean and green lentil

Table 2. The total carotenoids content in irradiated and non-irradiated chickpea, kidney bean and green lentil samples during storage. Mean value and standard error (n=3)

Table 3. Raffinose and stachyose content in irradiated and non-irradiated chickpea, kidney bean and green lentil samples during storage. Mean value and standard error (n=3)

Table 4. Thiamine and riboflavin content in irradiated and non-irradiated chickpea, kidney bean and green lentil samples during storage. Mean value and standard error (n=3)