EFFECT OF GAMMA IRRADIATION ON THE MICROBIAL LOAD, CHEMICAL AND SENSORY PROPERTIES OF KUBBA: PREPARED CHILLED MEAL

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Received on 14th November 2012
Revised on 16th May 2013

Locally prepared meal Kubba was subjected to gamma irradiation doses of 2, 4 or 6 kGy using gamma $^{60}$Co irradiator facility to prove safer products. Kubba meals were refrigerated (1-4°C). Microbiological, chemical and sensory characteristics of Kubba were evaluated at 0, 1, 2 and 3 weeks of storage. The results indicate that 4 and 6 kGy doses of gamma irradiation decreased the total viable (mesophilic aerobic) plate counts and increased the shelf-life of Kubba. Higher dose of gamma irradiation (6 kGy) decreased the protein and fats constituents of Kubba. The three chemical parameters, total acidity, lipid peroxide and volatile basic nitrogen, which were chosen as the indices of freshness, were all well within the acceptable limit for up to one week for Kubba treated with 0 and 2 kGy, and for up to 3 weeks at 1-4°C for samples treated with 4 and 6 kGy. Sensory evaluation showed no significant differences between irradiated and non-irradiated samples.

Keywords: Kubba, irradiation, refrigeration, sensorial evaluation, shelf-life

Introduction
In the developed and developing countries, there is a growth in the demand for convenience ready to cook/eat minimally processed meat products (Sweet et al., 2006; Hoz et al., 2008). The food industry is focused on manufacturing long-shelf-life ready-to-eat (RTE) products in domestic portions from processed blocks (Cabeza et al., 2009; Gil-Diaz et al., 2009). Syrian market offers several such ethnic ready to cook/heat meals including meat products like Sheesh tawog, Kabab, Mutton pices, Borak, and Kubba. The Syrian consumers have recently started using prepared meals which are prepared and marketed by local supermarkets. The Syrian food industry is traditionally dominated by Kubba. Indeed a Kubba is consumed not only in Syria, but also in neighboring countries. These products are marketed only in the frozen state, but freezing facilities are expensive, also freezing affects the texture of these products and freezing dose does not eliminate pathogens.
Therefore, storage of these products in chilled state would be of advantage. But in the chilled state such products have limited shelf-life (Sweet et al., 2006).

Irradiation of food is widely recognized and is now legally accepted in at least 51 countries with a maximum overall average of 10 kGy (IAEA, 2008). Irradiation, as a method of meat products preservation, has excellent potential in the elimination of pathogenic and spoilage microorganisms from meat and meat products (Mayer-Miebach et al., 2005; Badr, 2004; Satin, 2002).

One of the major concerns in irradiation meat and meat products, however, is its effects on meat and meat products quality, mainly because of free radical reaction resulting in the possibility of color change, lipid oxidation and odor generation, and consumer response to these quality changes are quite negative (Du et al., 2002).

There is abundant literature on the effects of ionizing radiation on meat (Sweet et al., 2006; 2007), meat products (Chouliara et al., 2006), and prepared meals (Irawati et al., 2007). However, there is a lack of studies on the effect of irradiation on the overall quality of ethnic Syrian meat preparations. Therefore, the objectives of this study were to investigate the use of gamma irradiation in order to improve the microbiological quality of Kubba, as precooked prepared meals, by extending their shelf-life at refrigeration temperature, while preserving the nutritional and sensorial characteristics.

Materials and methods

Preparation and formulation of Kubba

Kubba was prepared by a local caterer. No changes were made to the way in which the Kubba is usually prepared in this industry. Kubba has two parts, in which the outer layer consists of ground pre-boiled wheat (borgel) (600 g) mixed with minced beef (400g) and spices, allspice (8 g), black pepper (4 g), white pepper (6.6 g), onion (50 g) and salt (13.3 g). Outer layer was stuffed with precooked lamb (1000 g), onion (222 g), fat (55 g), pistachio (222 g) and spices (allspice (5.6 g), black pepper (2.8 g), white pepper (5.6 g), nutmeg (4.4 g), cumin (8.9 g) and salt (33.3 g). After preparing, Kubba products were fried in sunflower oil for 2 – 3 min. Eight pieces of precooked Kubba were placed on polystyrene trays covered with lids made of polyethylene film. The film thickness is 0.087 mm and sealed properly. Each tray of Kubba was considered as a replicate.

Treatments and analysis performed during storage

Samples from packed Kubba were exposed to gamma radiation as pasteurization process at doses of 2, 4 and 6 kGy in a $^{60}$CO package irradiator (dose rate of 730 Gy h$^{-1}$). The irradiation was performed at room temperature (15–20°C). The absorbed dose was determined using alcoholic chlorobenzene dosimeter. Ethanol chlorobenzene is prepared in our lab by mixing 24 ml chlorobenzene, 4ml distilled water, 0.04 ml acetone, 0.04 ml benzene to 100 ml ethanol. The absorbed dose is determined by the measurement of chloride ions or hydrogen ions by means of Oscillotitrator (OK-302/2, Radelkisz, Budapest, Hungary) (Cserep et al., 1971). For
each treatment, 20 trays of Kubba were allocated and all were stored at 1–4°C. Microbiological and chemical analyses were performed on controls and treated samples immediately after irradiation, and weekly throughout the storage period, which lasted 3 weeks. Sensory evaluation and proximate analyses were done within two days of irradiation.

**Microbiological evaluation**

Three replicates from each treatment, non-irradiated and irradiated, were aseptically opened, and 10 g of whole Kubba were transferred to a sterilized glass bottle containing 90 ml of sterile physiological water (9 g kg⁻¹ NaCl). The bottle was shaken to homogenize the sample. Further dilutions were made as far as 10⁻⁶ by AOAC method (AOAC, 2010). The media used for the microbiological study were nutrient agar (Oxoid, CM 325, UK) for the total viable (mesophilic aerobic) plate counts (TPCs) (48 h incubation at 30°C). A cut-off value of 10⁷ CFU g⁻¹ for TPCs (Ayres, 1960), was used for the unacceptable samples, and no further analyses were carried out when those indicator values were exceeded.

**Chemical analysis**

Approximately 150 g of Kubba were blended for 15 s in a laboratory blender, and were used in all chemical analysis. Each sample was homogenized and analyzed in triplicates, to determine moisture and ash (drying for 6 h at 105°C, and ashing for 4 h at 550°C), fat (as extractable component in Soxhlet apparatus), protein (as Kjeldahl nitrogen) using standard methods (AOAC, 2010).

**Total acidity**

The total acidity was obtained by a direct titration with (0.1 N) NaOH and phenolphthalein as an indicator (Egan et al., 1981). Ten grams of each sample were magnetically stirred in a total volume of 100 ml distilled water for 30 min and the mixture was afterwards filtered. Ten ml of the filtrate were titrated with (0.1 N) NaOH using 3 drops of a phenolphthalein indicator. The total acidity was calculated as ml of (0.1 N) NaOH 0.0090 g lactic acid.

**Lipid oxidation**

Lipid peroxidation in terms of g iodine/100 g fat of Kubba was determined by the modified method of Buege and Aust (1978). Kubba sample of 1g was placed in a 250 ml test flask and homogenized with 20 ml solution of acetic acid (50% acetic acid, 50% chloroform). The mixture was vortexed, incubated in a hot water bath at 50°C for 30 min, and the samples filtered. The filtrate was received into 0.5 ml of potassium iodide (50%), held in a dark place for 2 min. Distilled water 100 ml, and 3 drops of starch 1% as an indicator were added, and the mixture was titrated by sodium thiosulfate- pentahydrate (0.01 N), added drop wise until the end point.

**Total volatile basic nitrogen (VBN)**

A sample (10 g) of Kubba was minced with 100 ml distilled water and washed into distillation flask with 100 ml distilled water, then 2 g of magnesium oxide and an antifoaming agent were added. The mixture was distilled using the microKjeldahl
distillation apparatus. Distillate was collected for 25 min into 25 ml 4% boric acid and 5 drops of Tashero indicator. The solution was titrated by (0.1 N) HCl to calculate the total volatile basic nitrogen in the sample in terms of mg VBN/kg Kubba (ppm) (Pearson, 1978).

Sensory evaluation
The sensorial criteria, especially taste, odor, color and texture of the irradiated and non-irradiated Kubba, were evaluated within two days of irradiation. Each panelist received four coded pieces of samples (one non-irradiated and three irradiated samples; one at each dose). All Kubba were tasted by 25 persons. Before testing, Kubba products were fried in sunflower oil for 5 min. Each member independently evaluated the Kubba samples for taste, odor, color and texture on a 5-point hedonic scale (1: extremely poor, 2: poor, 3: acceptable, 4: good, 5: excellent), according to Al-Bachir et al method (Al-Bachir et al., 2010).

Statistical analysis
The four treatments were distributed in a completely randomized design with three replicates. The data were subjected to the analysis of variance test (ANOVA) using the SUPERANOVA computer package (Abacus Concepts Inc, Berkeley, CA, USA; 1998). A separation test on treatment means was conducted using Fisher’s least significant differences (LSD) methods (Snedecor and Cochran, 1988) at 95% confidence level.

Results and discussion
Kubba characteristics
The proximate chemical compositions of non-irradiated and irradiated Kubba samples are presented in Table 1. Non-irradiated Kubba products contained 52.73±0.43 % moisture, while the percentages of crude protein, total lipids and ash were 9.88±0.75 %, 12.16 ± 0.10 %, and 1.93±0.06%, respectively. In general, a decreasing trend was observed in protein and lipid content with the higher irradiation doses. There were significant (p >0.05) differences in protein and lipid contents between the non-irradiated and the samples irradiated with 6 kGy.

Table 1. Effect of gamma irradiation on moisture, protein, fat and ash contents of Kubba (%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kGy</td>
<td>52.7±0.43b</td>
<td>9.9±0.75b</td>
<td>12.2±0.10b</td>
<td>1.9±0.06b</td>
</tr>
<tr>
<td>2 kGy</td>
<td>51.1±1.52ab</td>
<td>9.6±0.47ab</td>
<td>12.1±0.67b</td>
<td>1.9±0.02a</td>
</tr>
<tr>
<td>4 kGy</td>
<td>50.5±0.26a</td>
<td>9.7±0.59ab</td>
<td>10.2±0.40a</td>
<td>1.9±0.04b</td>
</tr>
<tr>
<td>6 kGy</td>
<td>50.4±1.24a</td>
<td>8.8±0.38a</td>
<td>9.3±0.53a</td>
<td>2.0±0.01b</td>
</tr>
<tr>
<td>LSD</td>
<td>1.9</td>
<td>1.1</td>
<td>0.89</td>
<td>0.07</td>
</tr>
</tbody>
</table>

1 Values within a column followed by the same letters are not significantly different at 0.05 significant level
From each treatment three replicates (n = 3).
Lipids are reported to be the most sensitive food components to the irradiation process (Venugopal et al., 1999). There is a relationship between the decrease of protein contents of Kubba and the total volatile basic nitrogen (VBN) due to irradiation (Table 3). The increase of the VBN is related to protein breakdown (Egan et al., 1981).

**Microbiological quality of irradiated Kubba**

As with all prepared meal containing raw or semi-raw meat, non-irradiated Kubba samples were found to be contaminated with relatively high initial counts of aerobic mesophilic microorganisms as their mean log10 counts reached 2.96 (Table 2). Short heat treatment, through preparation of Kubba stuff and frying the whole Kubba in oil, is not sufficient to eliminate all mesophilic bacteria, because the raw materials (beef meat, lamb meat and spices) have a high number of contents and the local environmental conditions are suitable to support the rapid growth of such contaminants.

Data in Table 2 indicate that 2, 4 and 6 kGy doses of gamma irradiation significantly (p<0.05) decreased the total (mesophilic aerobic) plate microorganism counts (TPCs) of Kubba compared to control. A reduction in the TPCs values as a result of irradiation at time 0 was found in samples in which the control was about 10^3 C/g. The reduction was a log cycle of more than 1 or 2 for 4 and 6 kGy, respectively. However, control and samples treated with 2 kGy reached the generally accepted spoilage number of microorganism counts 10^7/g (Ayres, 1960), after one week of storage. Meanwhile, treated Kubba with 4 or 6 kGy did not reach the same number after 3 weeks, and those samples were of a satisfactory microbiological quality. Thus the microbiological shelf-life of Kubba was significantly extended from less than one week (control) to more than 3 weeks (samples treated with 4 or 6 kGy).

**Table 2.** Effect of gamma irradiation on microbial load of Kubba stored at 1-4°C (log CFU/g)

<table>
<thead>
<tr>
<th>Storage period (Weeks)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>3.0±0.04a</td>
<td></td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2 kGy</td>
<td>2.2±0.18ab</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>4 kGy</td>
<td>1.7±0.30b</td>
<td>1.5±0.45a</td>
<td>0.97±0.85a</td>
<td>3.9±0.01a</td>
</tr>
<tr>
<td>6 kGy</td>
<td>0.930.81b</td>
<td>0.9±0.81a</td>
<td>1.00±0.00a</td>
<td>2.2±0.21b</td>
</tr>
<tr>
<td>LSD</td>
<td>0.83</td>
<td>1.49</td>
<td>1.37</td>
<td>0.65</td>
</tr>
</tbody>
</table>

1 Values within a column followed by the same letters are not significantly different at 0.05 significant levels.
2 R= Rejected

From each treatment three replicates (n = 3).

The effectiveness of irradiation in delaying spoilage of foods was reviewed by Olson (Olson, 1998). Our results are in agreement with the results reported on other prepared meal products. Those results indicated that irradiation with 4 or 6 kGy and storage under refrigeration (5°C) reduced the total microorganisms count and
increased the shelf-life of ground beef (Mohamed et al., 2011), Sheesh Tawoq (Al-Bachir, 2010), Chicken Kabab (Al-Bachir et al., 2010), corned beef (Sallam et al., 2000), chicken vegetable and chicken sweet corn soup (Irawati et al., 2007), mutton shami Kababs and pork salami (Sweet et al., 2005), Sausage production (Chouliara et al., 2006), Borak (Al-Bachir, 2007), luncheon meat (Al-Bachir, 2005), and Camel meat (Al-Bachir and Zeino, 2009).

**Chemical quality of irradiated Kubba**

**Total acidity**

Table 3 shows that, immediately after treatment, all used doses (2, 4 and 6 kGy) had no effect on total acidity of Kubba. A previous study indicates that, immediately after treatment, all used doses of gamma irradiation (2, 4, and 6 kGy) had no significant effect on total acidity of Borak as Syrian prepared meals (Al-Bachir 2007). The results are in agreement with those of King et al. (1998), who reported no differences for the free fatty acids on day 0 of storage between the non-irradiated and irradiated beef, trout and pork at dose up to 3.5 kGy. On the other hand Kanatt, et al. (1997) indicated that free fatty acid content (FFA) in meat decreased after irradiation. Throughout storage periods, the total acidity of both irradiated and non-irradiated Kubba increased. The increase was higher in the control than of irradiated samples. After one week of storage, 4 and 6 kGy doses of gamma irradiation significantly (p<0.05) decreased the total acidity. The amount of lactic acid in irradiated Nham was found to be lower than the amount in the non-irradiated samples at the same period of storage (Prachasithisak and Bunnak, 1994).

**Lipid oxidation**

Effects of gamma irradiation on lipid oxidation of Kubba were compared (Table 3). Immediately after treatment, lipid oxidation values for irradiated Kubba were not different from those of non-irradiated controls. The chemical changes in irradiated meat are initiated by the free radicals produced by irradiation (Ahn et al., 2002). Lipid is reported to be the most sensitive food component to the irradiation process (Venugopal et al., 1999). In the current study, however, the lipid of the samples was not notably affected by irradiation treatment. This may be attributed to packaging Kubba with polyethylene film preventing air and oxygen to pass through. Nam and Ahn (2003) reported that lipid oxidation of irradiated meat was the highest with aerobic packaging, the lowest with vacuum-packaging and in the middle with double-packaging. During storage, lipid oxidation values of irradiated and non-irradiated Kubba samples tended to increase. After one week of storage, in Kubba gamma irradiated with doses of 4 and 6 kGy, lipid oxidation significantly (p<0.05) increased. As the storage time increased, overall lipid oxidation increased, and the rate of lipid oxidation was faster in irradiated than non-irradiated beef (Nam and Ahn, 2003; Chae et al., 2009).

**Volatile basic nitrogen VBN**

There was an interaction between treatment and storage time on the VBN (Table 3). Immediately after treatment, the values of VBN of irradiated Kubba with 2 and 4
kGy doses of gamma irradiation were significantly (p<0.05) higher than those of the control. Previous studies in our lab indicated that VBN of irradiated Camel meat tend to increase (Al-Bachir and Zeino, 2009). The results, in general, are in good agreement with those of Kim et al. (2002) who found that irradiated meats produced new volatiles not found in non-irradiated meats (turkey, pork and beef) and the amounts of total volatiles were higher than in non-irradiated samples. However, refrigerated storage significantly increased (p<0.05) the VBN contents in the control samples of Kubba. These results agree with previous observations (Due et al., 2003). The TVN is related to protein breakdown (Egan et al., 1981) and the observed increases may be attributed to the formation of ammonia or other basic compounds due to the microbial activity (Banwart, 1981). After one week of storage, the values of VBN of irradiated Kubba with 2, 4 and 6 kGy were significantly lower than those of the control. Used doses of gamma irradiation decreased the rate of TVN formation during storage by reducing the initial levels of the common spoilage microorganisms (Table 2).

Table 3. Effect of gamma irradiation on Total acidity (%Lactic acid), lipid peroxide (g iodine/100g fat) and Volatile basic nitrogen (VBN) (ppm.), of kubba

<table>
<thead>
<tr>
<th>Storage period (Weeks)</th>
<th>Dose (Kgy)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total acidity (%Lactic acid)</td>
<td>0 kGy</td>
<td>0.18±0.01a1</td>
<td>0.317±0.030a</td>
<td>R2</td>
<td>R</td>
</tr>
<tr>
<td>2 kGy</td>
<td>0.16±0.01a</td>
<td>0.244±0.024a</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>4 kGy</td>
<td>0.18±0.00a</td>
<td>0.202±0.031b</td>
<td>0.222±0.054a</td>
<td>0.236±0.052a</td>
<td></td>
</tr>
<tr>
<td>6 kGy</td>
<td>0.17±0.02a</td>
<td>0.224±0.009b</td>
<td>0.244±0.036a</td>
<td>0.211±0.005a</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>0.03</td>
<td>0.047</td>
<td>0.104</td>
<td>0.084</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipid peroxide (g iodine 100 g fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kGy</td>
</tr>
<tr>
<td>2 kGy</td>
</tr>
<tr>
<td>4 kGy</td>
</tr>
<tr>
<td>6 kGy</td>
</tr>
<tr>
<td>LSD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volatile basic nitrogen (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kGy</td>
</tr>
<tr>
<td>2 kGy</td>
</tr>
<tr>
<td>4 kGy</td>
</tr>
<tr>
<td>6 kGy</td>
</tr>
<tr>
<td>LSD</td>
</tr>
</tbody>
</table>

1Values within a column followed by the same letters are not significantly different at 0.05 significant level
2R= Reject
From each treatment three replicates (n = 3)

Sensory quality of irradiated Kubba

Table 4 illustrates the results of the initial sensory evaluation carried out for the Kubba products. It was found that immediately after irradiation the overall sensory scores of irradiated and non-irradiated samples were not significantly (p<0.05)
different. Taste, odor, color and texture of irradiated samples were not different from its non-irradiated control and all the samples were acceptable. This observation is in agreement with different authors (Al-Bachir et al., 2010; Benedito et al., 2011). However, some reports (Lee and Ahn, 2005; Rababah et al., 2010) indicate that irradiation of meat can produce change in the aroma, color, and flavor that significantly affect consumer acceptance. A correlation between sensory evaluation and chemical parameters (total acidity and lipid oxidation) was observed in relation to irradiated Kubba. Peter et al. (1998) observed that the sensory changes were attributed to an increase in lipid oxidation due to exposure to oxygen during irradiation. Properly sealed packaging was highly proven to be effective in reducing taste, odor, color and texture problems in Kubba. The color and odor changes in irradiated meats are highly dependent upon packaging condition (Nam and Ahn, 2003).

Table 4. Effect of gamma irradiation on the taste, texture, color and flavor of kubba

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Taste</th>
<th>Flavor</th>
<th>Color</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kGy</td>
<td>4.0±0.95a²</td>
<td>4.3±0.97a</td>
<td>4.3±1.20a</td>
<td>3.8±1.3a</td>
</tr>
<tr>
<td>2 kGy</td>
<td>4.0±0.85a</td>
<td>4.3±0.75a</td>
<td>4.5±0.82a</td>
<td>3.8±1.0a</td>
</tr>
<tr>
<td>4 kGy</td>
<td>4.1±0.90a</td>
<td>4.1±0.79a</td>
<td>4.6±0.51a</td>
<td>4.3±1.0a</td>
</tr>
<tr>
<td>6 kGy</td>
<td>4.1±0.79a</td>
<td>3.8±1.20a</td>
<td>4.3±0.91a</td>
<td>4.3±0.8a</td>
</tr>
<tr>
<td>LSD</td>
<td>0.72</td>
<td>0.78</td>
<td>0.77</td>
<td>0.84</td>
</tr>
</tbody>
</table>

¹Data represent a 5 point scale ranging from 1 (very bad) to 5 (very good).
²Values within a column followed by the same letters are not significantly different at 0.05 significant level

Conclusion

In conclusion, irradiation doses of 4 and 6 kGy can be effective to control microorganisms in Kubba, with extending their refrigerated shelf-life for more than 3 weeks without any significant effects on chemical and sensory quality of the Kubba.

Acknowledgements

The author wishes to express his deep appreciation to the Director General of AECS. Partial support of this work by the International Atomic Energy Agency under the Research Contract No. 11910 is gratefully acknowledged. Additional financial support was obtained from the Syrian Atomic Energy Commission.

References


