

ORIGINAL RESEARCH PAPER

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

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Locally prepared meal *Kubba* was subjected to gamma irradiation doses of 2, 4 or 6 kGy using gamma ⁶⁰Co irradiator facility to produce 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 fat 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, sensory 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 tawoq*, *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 ⁶⁰Co 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* (%)

Treatment	Moisture	Protein	Fat	Ash
0 kGy	$52.7 \pm 0.43b^1$	$9.9 \pm 0.75b$	$12.2 \pm 0.10b$	$1.9 \pm 0.06b$
2 kGy	$51.1 \pm 1.52ab$	$9.6 \pm 0.47ab$	$12.1 \pm 0.67b$	$1.9 \pm 0.02a$
4 kGy	$50.5 \pm 0.26a$	$9.7 \pm 0.59ab$	$10.2 \pm 0.40a$	$1.9 \pm 0.04b$
6 kGy	$50.4 \pm 1.24a$	$8.8 \pm 0.38a$	$9.3 \pm 0.53a$	$2.0 \pm 0.01b$
LSD	1.9	1.1	0.89	0.07

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)

Storage period (Weeks)	0	1	2	3
Treatment				
0 kGy	3.0±0.04a ¹	² R	R	R
2 kGy	2.2±0.18ab	R	R	R
4 kGy	1.7±0.30b	1.5±0.45a	0.97±0.85a	3.9±0.01a
6 kGy	0.93±0.81b	0.9±0.81a	1.00±0.00a	2.2±0.21b
LSD	0.83	1.49	1.37	0.65

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

²R= 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 shammi *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 (Prachasitthisak 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*

Dose (KGy)	Storage period (Weeks)			
	0	1	2	3
Total acidity (%Lactic acid)				
0 kGy	0.18±0.01a ¹	0.317±0.030a	R ²	R
2 kGy	0.16±0.01a	0.244±0.024a	R	R
4 kGy	0.18±0.00a	0.202±0.031b	0.222±0.054a	0.236±0.052a
6 kGy	0.17±0.02a	0.224±0.009b	0.244±0.036a	0.211±0.005a
LSD	0.03	0.047	0.104	0.084
Lipid peroxide (g iodine 100 g fat)				
0kGy	0.035±0.001a ¹	0.042±0.006 ^a	R ²	R
2 kGy	0.038±0.008a	0.043±0.007 ^a	R	R
4kGy	0.045±0.02a	0.055±0.005b	0.064±0.05 a	0.065±0.011a
6kGy	0.038±0.005a	0.060±0.002b	0.068±0.02 a	0.078±0.023
LSD	0.023	0.010	0.008	0.041
Volatile basic nitrogen (ppm)				
0 kGy	76.0±7.0a ¹	119.0±1.0a	R ²	R
2 kGy	106.0±5.0b	62.3±1.5b	R	R
4 kGy	138.0±7.0c	77.0±8.0c	111.3±1.5a	123.0±11.0a
6 kGy	75.7±2.5a	72.3±10.5bc	101.3±7.5a	105.0±5.0a
LSD	10.7	12.6	12.3	19.4

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*¹

Treatments	Taste	Flavor	Color	Texture
0 kGy	4.0±0.95a ²	4.3±0.97a	4.3±1.20a	3.8±1.3a
2 kGy	4.0±0.85a	4.3±0.75a	4.5±0.82a	3.8±1.0a
4 kGy	4.1±0.90a	4.1±0.79a	4.6±0.51a	4.3±1.0a
6 kGy	4.1±0.79a	3.8±1.20a	4.3±0.91a	4.3±0.8a
LSD	0.72	0.78	0.77	0.84

¹ Data represent a 5 point scale ranging from 1 (very bad) to 5 (very good).

² Values within a column followed by the same letters are not significantly different at 0.05 significant level
n = 25 persons

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*.

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