

## The Effects of Gamma Irradiation on the Quality of Ready-to-Cook Meatballs

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**Abstract:** The effects of gamma irradiation on microbial quality, lipid oxidation, and sensory characteristics of meatballs were studied, and these effects were compared with the effects on ground beef. Meat samples were irradiated at 0, 3, 6, and 9 kGy, then stored at  $4 \pm 1$  °C for 14 days. Irradiation resulted in greater microbial inactivation, lower lipid oxidation, and less adverse effects on sensory characteristics in meatballs compared to ground beef. Irradiation of meatballs at 3 kGy resulted in about a 4-log reduction in total microbial count without significantly affecting the quality of the product during the first 3 days of storage. Higher doses or longer storage in air resulted in quality degradation, which could be controlled by proper modified atmosphere packaging (MAP). Thus, irradiation doses approved for ground beef can be safely used with meatballs without significant quality degradation. Further studies combining irradiation and MAP are needed to determine the shelf-life of irradiated meatballs.

**Key Words:** Irradiation, meatball, ground beef, lipid oxidation, sensory quality, microbial quality

### Gama Işınlarnın Pişirmeye Hazır Köftelerin Kalitesi Üzerine Etkileri

**Özet:** Bu çalışmada gama ışınlarının köftelerin mikrobiyolojik kalite, yağ oksidasyonu ve duysal kalitesi üzerine etkileri incelenmiş ve sonuçlar gama ışınlarının kıyma üzerindeki etkileri ile karşılaştırılmıştır. Köfte ve kıyma örnekleri 0, 3, 6 ve 9 kGy dozlarında ışlandıktan sonra  $4 \pm 1$  °C de 14 güne kadar depolanmıştır. Işınlama işlemi köftelerde kıyma ile karşılaştırıldığında daha fazla mikrobiyal inaktivasyona, daha düşük yağ oksidasyonuna ve duysal özelliklerde daha az değişime neden olmuştur. 3 kGy dozda ışınlama köftelerin mikrobiyal yükünde 4-log kadar azalmaya neden olurken ilk 3 günlük depolama esnasında köftelerin kalitesinde önemli bir değişime yol açmamıştır. Ancak 3 kGy'den yüksek dozlarda veya daha uzun süre depolamada köftelerin kalitesinde önemli düşüş gözlenmiştir. Köftelerdeki bu değişimler ışınlama işlemiyle beraber uygun modifiye atmosferlerde ambalajlama ile birleştirilerek kontrol edilebilir, dolayısıyla bu konuda çalışmalara ihtiyaç vardır.

**Anahtar Sözcükler:** Işınlama, köfte, kıyma, yağ oksidasyonu, duysal kalite, mikrobiyal kalite

### Introduction

Consumer demand for high quality convenient food products in Turkey has been increasing and this has led to an increase in the commercial production of ready-to-cook meat products. These products include pre-portioned and pre-marinated fresh beef and poultry products, hamburger patties, meatballs, and kebabs. Traditional Turkish meatballs are prepared from ground beef, bread crumbs, and a mixture of various herbs and spices, including onion, parsley, black pepper, red pepper, and cumin. Meatballs are consumed in large quantity and ready-to-cook packaged meatballs have been introduced

into the Turkish market. Ready-to-cook meat products can cause foodborne illnesses if they are not properly cooked. For instance, *Escherichia coli* O157:H7 is a potential pathogen in red meat and has been associated with a number of foodborne disease outbreaks (1). Preservation methods to maintain the quality and safety of the product for a longer period are needed.

Gamma irradiation is one of the most effective preservation methods to ensure the microbiological safety of meat products (2,3). The US Food and Drug Administration (FDA) approved irradiation of fresh chilled meat and meat products at doses of up to 4.5 kGy to

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eliminate pathogens (4). Doses up to 7 kGy have been approved for fresh and frozen meat products to control pathogens in Turkey (5). Although the microbial safety of meat products can be ensured by irradiation, quality degradation may occur due to irradiation-induced changes in flavor and color (6-8). These undesirable changes are oxidative in nature and can be inhibited by antioxidants (6). The herbs and spices present in meatballs may inhibit radiation-induced changes in quality and enhance microbial inactivation through their antioxidant and antimicrobial activity (6-8). Therefore, quality maintenance and shelf-life extension of meatballs can be achieved at lower irradiation doses compared to the doses used with ground beef.

The objective of this study was to determine the effects of gamma irradiation on microbial quality, lipid oxidation, and sensory characteristics of meatballs. The effects on meatballs were compared to the effects on ground beef.

## Materials and Methods

Ground beef (about 20% fat content), table salt, parsley, black pepper, cumin, red pepper, and onion were purchased from a local supermarket. Plate count agar (PCA) and peptone were purchased from Oxoid (Hampshire, UK), thiobarbituric acid (TBA) was purchased from Merck (Darmstadt, Germany), butylated hydroxytoluene (BHT), 1,1,3,3-tetraethoxypropane (TEP), and trichloroacetic acid (TCA) were purchased from Fluka (Buchs, Switzerland).

Meatballs were prepared in the laboratory with the following ingredients: ground beef (79.8%), onion (8%), bread crumbs (4.8%), parsley (3.2%), black pepper (1.6%), red pepper (0.35%), cumin (0.65%), and salt (1.6%). All ingredients were mixed and kneaded by hand for about 15 min. The ground beef samples were kneaded similarly without the addition of any other ingredients. Pre-weighed samples (25 g each) were aseptically placed in sterile stomacher bags for microbial analysis or were placed in plastic trays wrapped with a plastic film for TBA and sensory analyses. The samples were transported to the irradiation plant in refrigerated boxes at  $9 \pm 3$  °C.

Ground beef and meatball samples were irradiated at 3, 6, and 9 kGy doses with a  $^{60}\text{Co}$  source at  $9 \pm 3$  °C in a commercial food irradiation plant (Gamma-Pak GmbH.,

Çerkezköy, Turkey). The irradiation treatments were performed twice. Absorbed doses were measured with Amber 3042 dosimeters (Harwell Dosimeters Ltd, Oxfordshire, UK). Non-irradiated (0 kGy) control samples were kept outside the irradiation chamber during the treatments. All samples were stored at  $4 \pm 1$  °C in aerobic conditions for up to 14 days.

Samples (25 g each) were homogenized in 225 ml of sterile peptone water (0.1%) using a stomacher (Seward 400, Seward Ltd, London, UK) at medium speed for 2 min. Appropriate dilutions were made and pour-plated in duplicate using PCA. The plates were incubated at 35 °C for 48 h and the colony forming units were counted.

Lipid oxidation was determined by the TBA method (9). A 10-g sample was mixed with 34 ml of TCA solution (5% in water) and 1 ml of BHT solution (7.2% in ethanol), and then homogenized using a blender (Waring Products Inc., Torrington, CT, USA) at high speed for 2 min. It was poured through filter paper (Whatman No. 4) into a 50-ml flask and diluted to the volume with the TCA solution. Next, 5 ml of this solution was mixed with 5 ml of 0.02 M TBA solution in a test tube and incubated at 80 °C in a water bath for 20 min to develop color. Absorbance at 532 nm was measured against a blank using a spectrophotometer (Philips PU 8625 UV/VIS, Cambridge, UK). A standard curve was obtained using TEP as the malonaldehyde standard at different concentrations. The thiobarbituric acid reactive substances (TBARS) value was calculated by multiplying the absorbance by the constant calculated from the standard curve and was expressed as mg of malonaldehyde (MDA) per kg of meat in the samples.

The triangle test was conducted by a sensory panel of 26-31 untrained panelists in a sensory lab to detect the radiation-induced changes in odor and color of the irradiated samples after 3- and 10-day storage. In each test the panelists were given 2 non-irradiated samples and 1 irradiated sample that were blindly coded with 3-digit numbers, then asked to detect the odd one considering odor or color. Thus, 3 sets of samples were used to differentiate the samples irradiated at 3, 6, and 9 kGy from the non-irradiated (0 kGy) sample, and another set was used to differentiate samples irradiated at 9 kGy from those irradiated at 3 kGy. Freshly prepared non-irradiated samples were used as a control in the test conducted 10 days after irradiation. The panelists were also asked to specify the difference in a few words and to

indicate the direction of the difference (desirable, undesirable, or neutral).

Analysis of variance (ANOVA) was performed to determine the effect of radiation dose and storage time on total aerobic mesophilic count and TBARS values using Minitab® v.12.2 (Minitab Inc., State College, PA, USA). Tukey's pair wise comparison was conducted to determine the differences among the treatments. The probability level for the triangle test was determined according to Roessler et al. (10). Bacterial population data were fitted to a regression equation ( $\text{Log}_{10} \text{CFU g}^{-1} = A \cdot \text{Dose} + B$ ) and the  $D_{10}$  value was determined as the negative slope of the equation ( $D_{10} = -A$ ).

## Results

Total aerobic mesophilic counts after 1-day post-irradiation storage at  $4 \pm 1$  °C decreased as irradiation dose increased up to 6 kGy (Table 1). No further reduction in the microbial count of ground beef was observed after increasing the dose from 6 to 9 kGy ( $P > 0.05$ ), while a significant additional reduction was obtained in meatballs ( $P < 0.05$ ). Irradiation at 6 and 9 kGy resulted in up to a 6-log reduction in total microbial counts, resulting in a final count of about 3-log after day 1. The total microbial count increased during storage, but it was lower in samples irradiated at higher doses (Table 1). The total count on day 14 in all meat samples irradiated at 6 and 9 kGy was lower than the count in non-irradiated samples on day 1, while the count on day 14 in samples irradiated at 3 kGy were similar to the

count in non-irradiated samples on day 1 (Table 1). Total aerobic mesophilic count reached very high levels ( $> 10^{12} \text{CFU g}^{-1}$ ) in non-irradiated samples during storage beyond 7 days. The  $D_{10}$  value in meatballs was 0.86 kGy, while it was 1.26 kGy in ground beef. Total aerobic mesophilic counts were lower in meatball samples than in ground beef on days 1, 7, and 14 (Table 1).

All irradiated samples had significantly greater TBARS values than non-irradiated samples (Figure A and B). Meatballs irradiated at 6 and 9 kGy had higher TBARS values than the non-irradiated meatballs; however, no significant difference was detected between the TBARS values of meatballs irradiated at 3, 6, and 9 kGy ( $P > 0.05$ ). Ground beef irradiated at 3 kGy had a lower TBARS value than that irradiated at 9 kGy. TBARS values for meatballs were up to 75% lower than for ground beef (Figure A and B). TBARS values of all samples increased during storage, but this increase was more pronounced in ground beef than in meatballs. Differences between the TBARS values of ground beef measured on day 1, day 7, and day 14 were significant ( $P < 0.05$ ). On the other hand, no significant increase in the TBARS values of meatballs was observed during 7-day storage. TBARS values in meatballs increased significantly after 14-day storage.

Irradiation at 3 kGy did not significantly change the odor of meatballs after 3-day storage (Table 2). Only 31% the panelists who successfully identified irradiated meatballs indicated that the meatballs irradiated at 3 kGy were undesirable on day 3. However, meatballs irradiated

Table 1. The effect of irradiation dose on the total aerobic mesophilic population ( $\log_{10} \text{CFU g}^{-1} \pm$  standard deviation) in ground beef and meatballs during storage at  $4 \pm 1$  °C.

Sample	Dose (kGy)	Day 1	Day 7	Day 14
Ground beef	0	9.97 ± 0.05 ax	TNTC ay	TNTC az
	3	5.70 ± 0.32 bx	7.51 ± 0.18 by	9.39 ± 0.13 bz
	6	2.99 ± 0.20 cx	5.16 ± 0.40 cy	7.57 ± 0.09 cz
	9	3.37 ± 0.22 cx	4.69 ± 0.13 cy	7.34 ± 0.11 cz
Meatballs	0	8.48 ± 0.01 ax	TNTC ay	TNTC az
	3	4.88 ± 0.09 bx	7.41 ± 0.05 by	8.89 ± 0.16 bz
	6	3.74 ± 0.13 cx	3.39 ± 0.17 cy	5.93 ± 0.17 cz
	9	3.19 ± 0.02 dx	3.44 ± 0.22 dy	5.43 ± 0.02 dz

Values represent the average of 2 observations ± standard deviation.

Different letters (a-c) in each column for ground beef and meatballs indicate significant difference ( $P < 0.05$ ).

Different letters (x-z) in each row indicate significant difference ( $P < 0.05$ ).

TNTC: Viable cells were too numerous to count,  $> 10^{12} \text{CFU g}^{-1}$ .

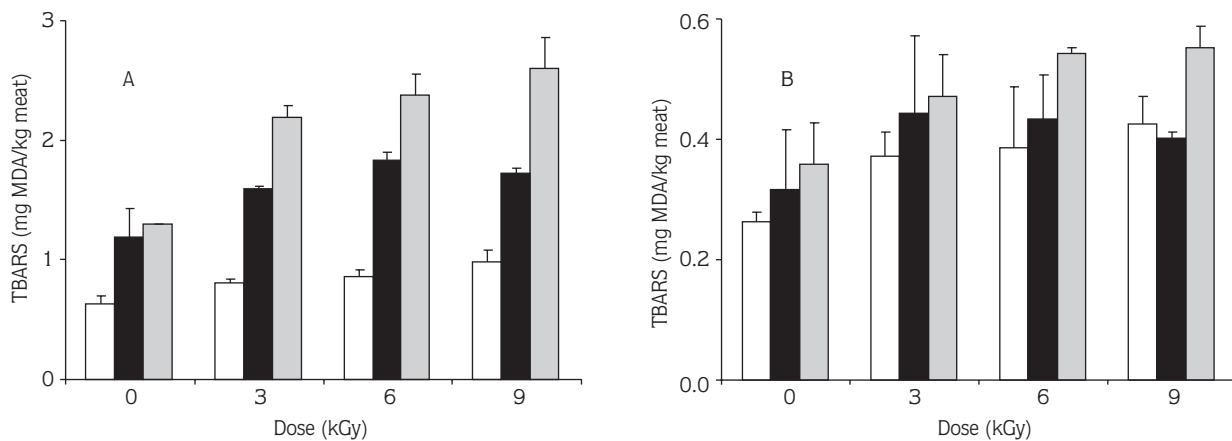


Figure. The effect of irradiation dose on the TBARS values of ground beef (A) and meatballs (B) during storage at  $4 \pm 1$  °C for 1 day (□), 7 days (■), and 14 days (▣). Error bars represent the standard deviation of the means (n = 2).

Table 2. Discrimination (triangle test) of the odor and color of irradiated samples from non-irradiated controls (0 kGy), and the direction of the difference.

Sample	Dose (kGy)	Storage time (day)	Total number of panelists	Number of correct answers	Direction of difference (%) <sup>a</sup>			Significance of difference	
					Desirable	Undesirable	Neutral		
ODOR	Ground beef	3	3	26	16	19	44	37	*
			10	31	26	23	50	27	*
		6	3	26	16	12	38	50	*
			10	31	28	18	50	32	*
		9	3	26	17	35	47	18	*
			10	31	27	15	59	26	*
	Meatballs	3	3	26	13	8	31	62	N.S.
			10	31	29	34	31	34	*
		6	3	26	21	10	52	38	*
			10	31	30	30	40	30	*
COLOR	Ground beef	3	3	26	16	19	44	37	*
			10	31	29	24	79	7	*
		6	3	26	17	18	24	58	*
			10	31	31	13	84	3	*
		9	3	26	17	29	53	18	*
			10	31	29	10	72	18	*
	Meatballs	3	3	26	16	6	25	69	*
			10	31	31	26	52	23	*
		6	3	26	19	11	53	37	*
			10	31	30	30	50	20	*
9	3	26	25	0	52	48	*		
	10	31	29	24	45	31	*		

<sup>a</sup>: Expressed as the % of correct answers; \*: significant (P < 0.05); N.S.: not significant.

at 6 and 9 kGy were successfully differentiated from the control (0 kGy) on day 3. Samples irradiated at 6 and 9 kGy were found to be undesirable by up to 63% of the

panelists (Table 2). The odor of all the irradiated meatballs was successfully distinguished from the freshly prepared control after 10-day storage. The difference

between meatballs irradiated at 3 and 9 kGy was also significant on days 3 and 10 (Table 3). Irradiation at all doses resulted in significant change in the odor of ground beef, as detected on day 3 and day 10 by the sensory panel (Table 2). About 40% of the successful panelists indicated that the odor of the irradiated ground beef was undesirable on day 3, and this percentage increased up to 60% on day 10 (Table 2). The difference between the odor of ground beef irradiated at 3 and 9 kGy was also significant (Table 3).

Irradiation at 3, 6, and 9 kGy resulted in significant changes in the color of meatballs and ground beef (Table 2). The change in color increased during storage. Only 25% of the panelists who successfully discriminated the irradiated meatballs at 3 kGy indicated that the color of the samples on day 3 was undesirable. This fraction increased to 52% on day 10. On the other hand, the color of ground beef irradiated at 3 kGy was found to be undesirable by 44% and 79% of the panelists on day 3 and day 10, respectively. As the irradiation dose increased, more undesirable changes in color were detected (Table 2). The difference between the color of ground beef irradiated at 3 and 9 kGy was significant on days 3 and 10 (Table 3).

## Discussion

We found greater irradiation-induced microbial inactivation in the meatballs than in the ground beef, possibly due to the antimicrobial effects of the spices used

in the meatballs. Spices present in meatballs decreased the growth rate of microorganisms during storage. Similar reductions in total aerobic mesophilic counts in ground beef and patties upon irradiation have been reported by other researchers (11,12). Antimicrobial activity of spices has been reported in several studies (7,13); however, it is important that the spices used be free of pathogens and have low-level microbial contamination.

Lipid oxidation in meat produces off-flavor and limits the shelf life of meat products (14). Irradiation of fresh meat products results in oxidative rancidity (14), which limits the maximum applicable irradiation dose to inactivate pathogenic microorganisms. Use of antioxidants, herbs, and spices inhibited lipid oxidation in meat products (6,15). In the present study irradiation-induced lipid oxidation in meatballs was much lower than that in ground beef. We used parsley, black pepper, red pepper, and cumin in preparation of meatballs, and these ingredients decreased radiation-induced lipid oxidation. The antioxidant activity of these herbs and spices have been reported in a number of studies (16,17). Lipid oxidation in irradiated meat products is also related to the oxygen concentration (18); therefore, O<sub>2</sub> concentration in the package atmosphere can be reduced during irradiation treatment and the storage period to reduce lipid oxidation in the products. Moreover, CO<sub>2</sub>-rich MAP application might have advantages, from the microbial point of view, and thus needs to be investigated.

Table 3. Discrimination (triangle test) of the odor and color of samples irradiated at 9 kGy from samples irradiated at 3kGy, and the direction of the difference.

	Sample	Storage time (day)	Total number of panelists	Number of correct answers	Direction of difference (%) <sup>a</sup>			Significance of difference
					Desirable	Undesirable	Neutral	
ODOR	Ground beef	3	26	14	21	43	36	*
		10	31	18	11	67	22	*
	Meatballs	3	26	15	0	53	47	*
		10	31	16	31	25	44	*
COLOR	Ground beef	3	26	17	12	35	53	*
		10	31	20	15	15	70	*
	Meatballs	3	26	18	6	50	44	*
		10	31	11	27	18	55	N.S.

<sup>a</sup>: Expressed as the % of correct answers; \*: significant (P < 0.05); N.S.: not significant.

The odor and color differences observed in our study due to irradiation is similar to those observed in other studies (15,19). Change in odor and color was less pronounced in meatballs than in ground beef. Herbs and spices in meatball samples led to a dark brown color, which darkened further (slightly) upon irradiation. On the other hand, irradiation resulted in significant loss of the bright red color of ground beef, which was easily detected by the panelists. The insignificant changes in odor and color of irradiated (3 kGy) meatballs during the first 3 days of storage indicates that this dose level could be used without quality degradation in meatballs

In conclusion, irradiation resulted in greater microbial inactivation, lower lipid oxidation, and less adverse effects on sensory characteristics in meatballs than in ground beef. The herbs and spices present in meatballs inhibited radiation-induced degradation in quality and enhanced inactivation of microorganisms due to irradiation. Thus,

irradiation doses approved for ground beef can be safely used for meatballs without significant quality degradation. Irradiation of meatballs at 3 kGy resulted in about a 4-log reduction in total microbial count without significantly affecting the quality of the product during the first 3 days of storage; however, quality degradation occurred during longer storage periods and at higher doses. The observed losses in quality could be controlled by a combination of MAP and irradiation, which needs to be investigated further.

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