Praca oryginalna *Original paper*

Effect of gamma irradiation on microbiological and physico-chemical quality of meatballs*)

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Summary

The aim of the study was to examine the effects of gamma irradiation on the microbiological and physio-chemical characteristics of meatballs. Meatball samples were produced experimentally and divided into six subgroups. The first group (control group) was not subjected to an irradiation process, and the remaining groups were subjected to irradiation at doses of 1, 2, 3, 5 and 7 kGy. All groups were analyzed on days 0 (the day of irradiation process), 5, 10, 15, 20 and 30.

The study revealed a significant reduction in the microbiological flora of the meatballs in relation to gradual increases in irradiation dosage. Faecal coliform bacteria counts, coliform bacteria counts and S. aureus counts were eliminated after applying 2 kGy, 3 kGy and 7 kGy irradiation doses, respectively. During storage at 4±1 °C, the bacterial populations systematically increased. Compared to the control group, low dose irradiation did not significantly change pH values, but higher pH levels were determined during high doses of irradiation. Irradiation increased the peroxide levels, but this increase did not seem to correlate with irradiation dosage. FFA levels decreased in relation to increasing levels of irradiation dosage.

Keywords: gamma irradiation, meatball, meat quality

Ionizing radiation is an effective method of preservation for fresh red meats (20). It can effectively eliminate potentially pathogenic bacteria including *Salmonella* and *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli O157:H7* or *Campylobacter* from suspected food products without affecting sensory and nutritional qualities (9).

Irradiation is a physical food protection method which provides prolonged protection and protecting food quality and safety (17). Pathogenic bacteria in foods were eliminated by applying the irradiation. This method provides various benefits, including providing hygienic quality, extending shelf life, preventing parasitic contamination, eliminating insects and pests during storage, preventing germination and advanced maturation and decreasing food losses (18, 19, 21).

Although there are differences in the ingredients between regions, the meatball is one of the most common consumed products in the catering and fast food sector. Because of ground meat, spices and other ingredients, uncooked meatball has a high microbiological load and short shelf life. The aim of this study was to examine the effects of gamma irradiation on the microbiological and physico-chemical characteristics of uncooked meatballs.

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Material and methods

Preparation and packaging of meatballs. Beef (74-75%) and lamb meat (10%) were used for meatball production. Meat contain 21% of fat and no extra fat was added. The meats were ground and homogenously mixed with 6.0% dried bread powder, 1.0% salt, 5.0% powdered onion, 0.5% black pepper, 0.5% cumin, 0.5% red pepper, 0.5% allspice and 0.6% sodium bicarbonate and 1.0% water. The meatballs were shaped (8.5 × 2 × 1 cm) and portioned to plastic plates (10 × 14 × 3 cm). Plates were wrapped with stretch film. Packed meatballs were labeled and divided batches.

Storage of meatballs. After storing of meatball samples during one night at 4 ± 1°C, they were transported using refrigerated transport means within two hours to irradiation facility and immediately irradiated. Meatball samples, exposed to irradiation, were stored in refrigerator at 4 ± 1°C until irradiation of all groups was completed. Upon completion of irradiation, all groups were transported back to the laboratory in refrigerated conditions not later than within two hours and analyses were initiated immediately. Unanalyzed samples were stored for in refrigerator at 4 ± 1°C.

Irradiation of meatballs. Irradiation was performed in Gamma-Pak Sterilization Company, which is founded in Çerkezköy. The irradiation facility was a Nordion-Canada model JS 9600 boxed irradiation device and had a source loading capacity up to 3.000.000 Ci.

Five batches, except non-irradiated (control), were subjected to gamma irradiation (Cobalt^{60}) at 15°C and at doses of 1, 2, 3, 5 and 7 kGy, respectively. The mean dose rate during the irradiation was determined as 1 kGy/hr.

Secondary standard Ceric-Cerious chemical dosimeters and Harwell red acrylic (Perspeks) (PMMA) dosimeters of plastic character were used as reference dosimeter for measuring the dose radiation absorbed by the irradiated products. For validation and process control, Harwell red acrylic dosimeters which can be calibrated against secondary standard Ceric-Cerious dosimeters were used as daily dosimeter.

(The authors would like to thank Dr. Hasan Alkan from Gamma-Pak Sterilization Company).

Microbiological analysis. All procedures, including sampling, preparation of growth media, sterilization, homogenization, dilution and enumeration were performed according to methods specified in Turkish Standards Institute (29-31). 10 grams of each meatball samples were taken and homogenized with 90 ml of peptone water (Oxoid, Hampshire,UK) in a stomacher. Serial decimal dilutions were made using the same media and then plated in duplicate for bacterial counts. Standart media and procedures were used (1-3, 12). Total aerobic mesophilic bacteria (TAMB) in Plate Count Agar (Oxoid CM325) after 48 h at 35°C, Total aerobic psychrophilic bacteria (TAPB) in Plate Count Agar (Oxoid CM325) after 14 days at 4°C, Coliform bacteria in Violet Red Bile Agar (Oxoid CM107) after 24 h at 35°C, Faecal coliform bacteria in Violet Red Bile Agar (Oxoid CM107) after 24 h at 44°C, *Staphylococcus aureus* in Baird Parker Agar (Oxoid CM275) after 48 h at 35°C, Lactic acid bacteria (LAB) in Man Rogosa Sharpe Agar (Oxoid CM361) after 48 h at 35°C, Mould and yeast counts were determined in Potato Dextrose Agar (Oxoid CM139) after five days at 25°C.

Physico-chemical analysis. Determinations of moisture, fat, pH, peroxide value (PV) and free fatty acids (FFA) were performed according to the methods, which are described in AOAC (5), TS 1605 (26), TS 3136 (27), and TS 4964 (28) respectively.

Statistical analysis. Production of experimental meatball, irradiation and all analyses were repeated in triplicate. All analyses were performed in duplicate and the results obtained were evaluated statistically. SPSS programme for Windows (25) was used in formation of statistics and variance analysis method (ANOVA) was utilized. Duncan multiple analysis method was used to determine the differences between batches ($p < 0.05$).

Results and discussion

Results of microorganism numbers detected in the non-irradiated (control) group and in irradiated samples during 30 day storage at 4°C are provided in figure 1, 2, 3, 4, 5, 6 and 7. Non-irradiated meatballs in the control group were found to have: Total Aerobic Mesophyllic Bacteria (TAMB) number of 8.21 ± 0.582 log cfu/g, Total Aerobic Psychrophillic Bacteria (TAPB) number 7.62 ± 0.851 log cfu/g, coliform bacteria count 3.77 ± 0.378 log cfu/g, faecal coliform bacteria number 3.29 ± 0.358 log cfu/g, *S. aureus* number 5.53 ± 0.709 log cfu/g, lactic acid bacteria 6.40 ± 0.501 log cfu/g, mold and yeast 6.70 ± 0.355 log cfu/g. These numbers displayed significant reduction depending on the dose of irradiation and at doses of 1, 2, 3, 5 and 7 kGy, the values were found to be 6.36, 5.18, 4.28, 3.24 and 2.24 log cfu/g for TAMB; 5.38, 4.62, 3.71, 2.72 and < 1.3 log cfu/g respectively for TAPB; 4.43, 2.83, 2.56, 1.80 and < 1.3 log cfu/g respectively for *S. aureus*; 4.52, 3.98, 3.19, 2.01 and 1.41 log cfu/g respectively for Lactic acid bacteria; 5.13, 3.84, 3.26, 2.07 and 1.32 respectively for molds and yeasts. The number of coliform bacteria was 2.66 log cfu/g at 1 kGy irradiation dose, and 1.54 log cfu/g at 2 kGy irradiation dose, and dropped below countable amount at doses of 3 kGy and higher. The number of fecal coliform bacteria was found to be 1.85 log cfu/g at an irradiation dose of 1 kGy and it was detected under countable levels at doses of 2.0 kGy and more (higher). Bacterial populations grew regularly during the period of storage at 4 ± 1°C.

Various authors have found a high number of TAMB in meatball samples. Kaymaz (16) found a TAMB number of 1.3×10^{10} cfu/g in hamburger meatballs. Soyutemiz & Anar (24) detected a number of 3.6×10^{7} cfu/g in grill meatballs, whereas Çetin & Yücel (7) found a TAMB of 1.1×10^{6} cfu/g in butcher-made meatballs. These findings are, in terms of TAMB, similar to the findings in our study. It is likely that a high level of TAMB is due to contaminated ingredients. Particularly, the ground meat and spices have a considerable effect on microbial flora of meatballs.

Another objective of our study was to define the effect of irradiation on microflora, and therefore, the reduction due to doses of irradiation was taken as basis rather than the high level of baseline microflora. A decrease in TAMB number was observed with increasing doses. Exposure to a dose of 2 kGy resulted in a 3 log reduction. Irradiation at 7 kGy yielded a 6 log reduction in TAMB number. An approximate reduction by 3 log observed in our study in TAMB number following irradiation at 2 kGy was found consistent with studies of various authors on similar foodstuffs (15, 21, 22). In meatballs studies of Aytaç, Vural, & Özbaş (6) they reported that following irradiation at 1.5 kGy and 3.0 kGy, TAMB number was reduced by about 0.7 log and 1 log, respectively, there was no significant difference in TMAB at the end of 7 days of storage at 4°C. Dempster, Hawrysh, Shand, Lahola-Chomiak, & Corletto (8) found a reduction by 82% and 92%, respectively, in total bacteria count, in gamma irradiated beef burgers at doses of 1.03 kGy and 1.54 kGy.

Although there was an insignificant difference in terms of post-irradiation TAMB number between groups exposed to 2 and 3 kGy, 3 and 5 kGy and 5 and 7 kGy, there was a statistically significant difference between all other groups ($p < 0.05$).

Rodriguez, Lasta, Mallo, & Marchevsky (23) reported that the number of psychrophylic bacteria in beef

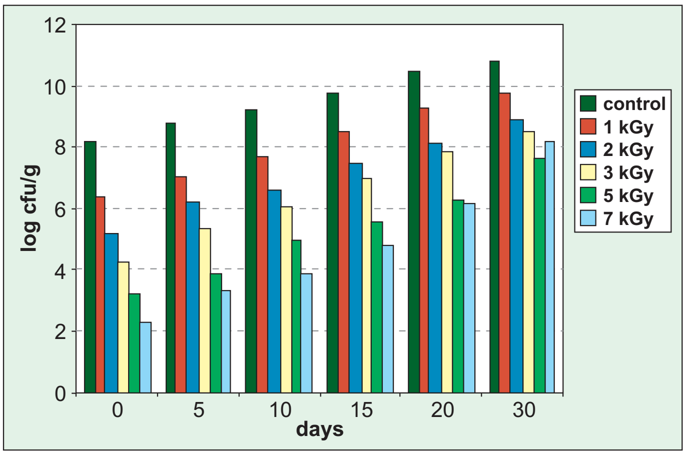

Fig. 1. TAMB counts

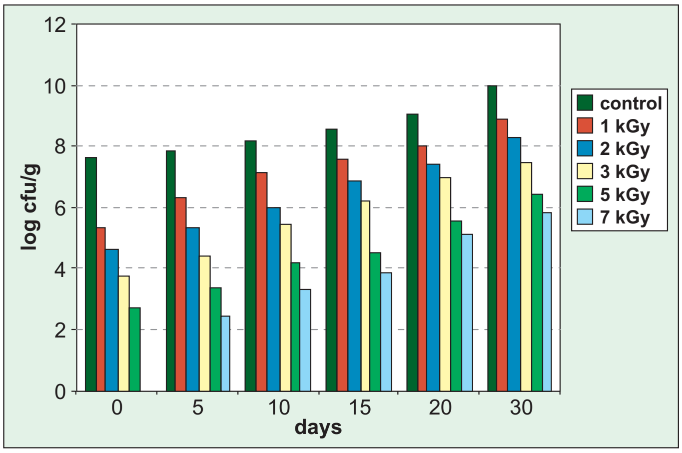

Fig. 2. TAPB counts

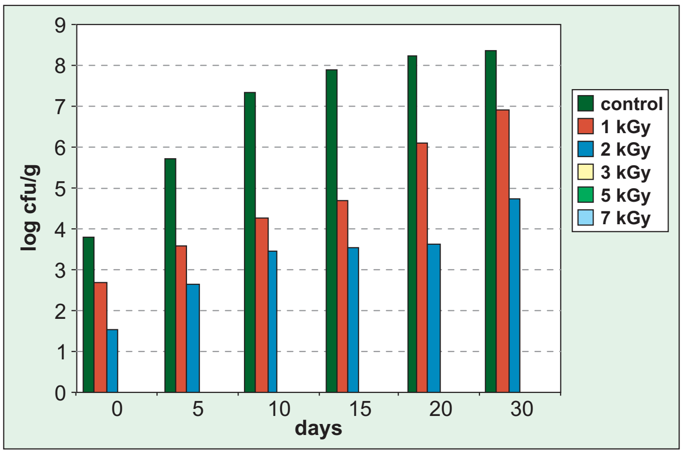

Fig. 3. Coliform counts

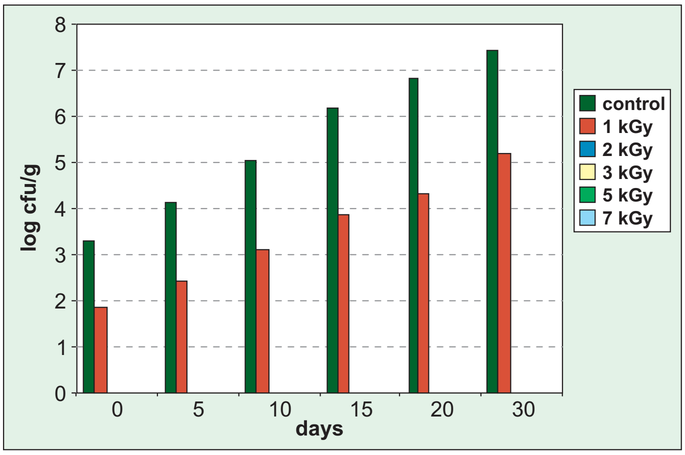

Fig. 4. Faecal Coliform counts

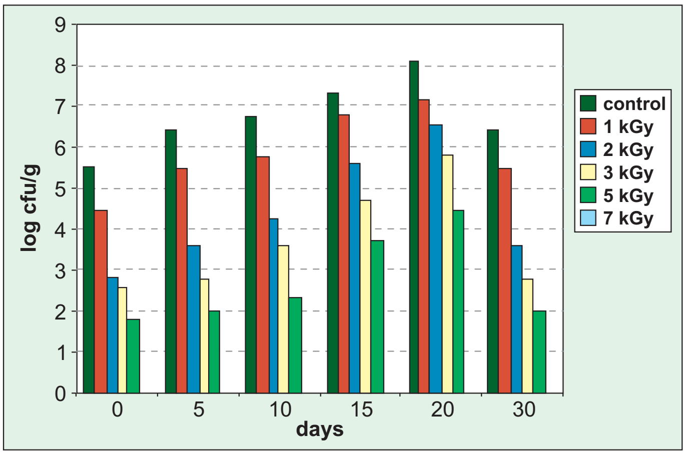

Fig. 5. *S. aureus* counts

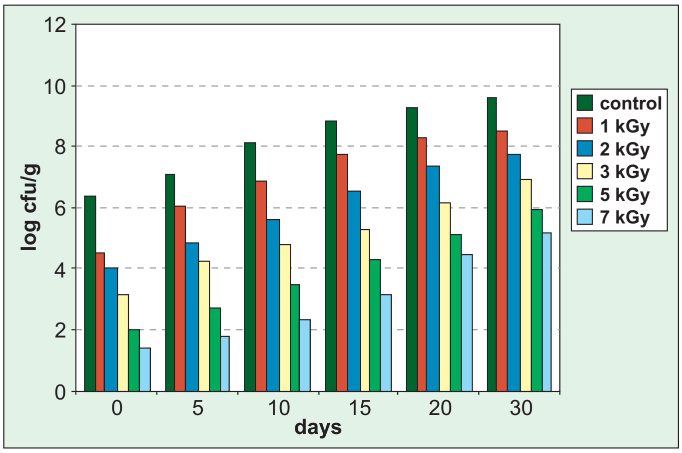

Fig. 6. LAB counts

in the control group reduced from 4.11 log cfu/cm^2 to < 1.58 log cfu/cm^2 following an irradiation at 2 kGy. This reduction is almost identical to 3 log reduction we have obtained with 2 kGy and support our findings. The difference between all batches were found to be statistically significant in terms of TPAB number, excluding groups exposed to 1 and 2 kGy irradiation ($p < 0.05$).

In our study we found that the baseline coliform bacteria was reduced to the level of 2.23 log following an irradiation of 2 kGy. This result is consistent with the results of Rodriguez, Lasta, Mallo, & Marchevsky (23). Kanatt, Paul, D'Souza, & Thomas (15) reported that the number of baseline faecal coliform bacteria in beef, which was 3.25 log cfu/g was totally eliminated following an irradiation of 2.5 kGy. This finding supports the findings in our study.

Naik, Paul, Chawla, Sherikar, & Nair (22) reported that 3.42 log cfu/g *Staphylococcus* spp. in the control group was reduced to undetectable levels following exposure to 2.5 kGy irradiation, and Kanatt, Paul, D'Souza, & Thomas (15) had a similar finding regar-

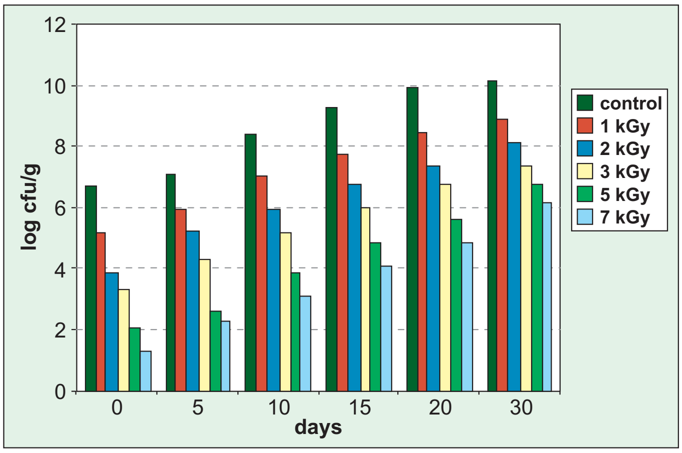

Fig. 7. Mould and yeast counts

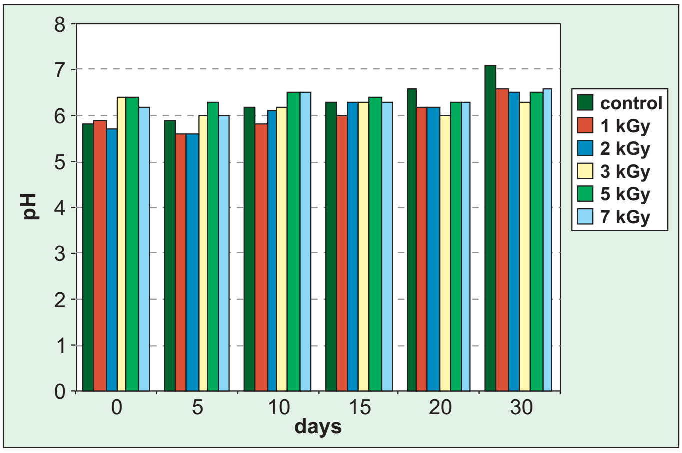

Fig. 8. pH values

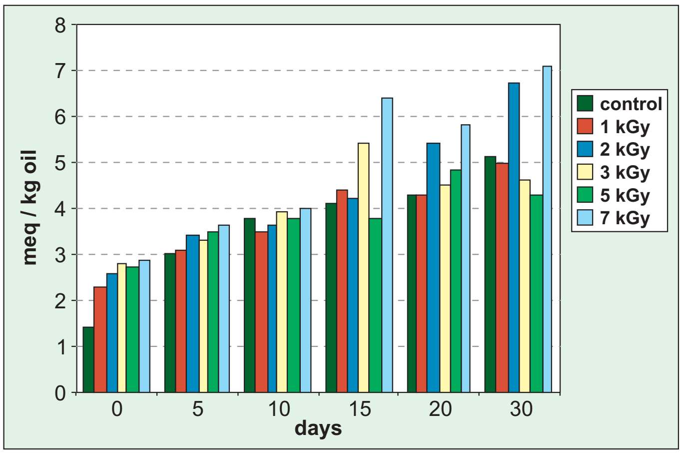

Fig. 9. Peroxide value

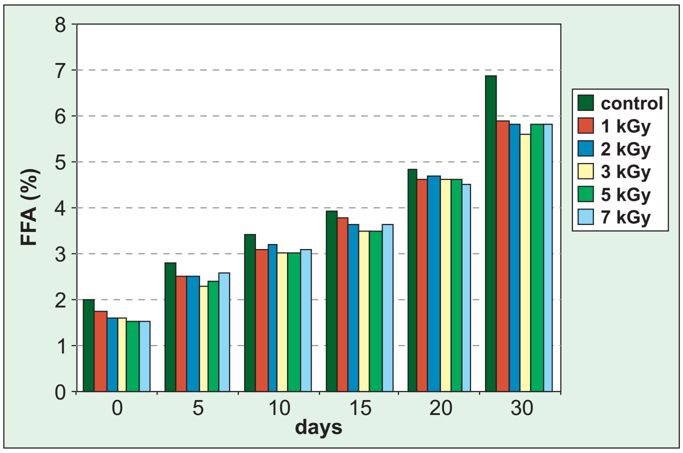

Fig. 10. Free fatty acids (%)

ding *Staphylococcus* spp. existing at 4.54 log cfu/g level in beef which were totally eliminated following irradiation at the same dose. In this study, reduction of *S. aureus* to undetectable levels was achieved only at 7 kGy irradiation dose. This was considered to be due to the number of baseline *S. aureus* being higher than those in the above mentioned two studies (15, 22). Following irradiation, groups exposed to 2 and 3 kGy, and 3 and 5 kGy, the difference in the number of *S. aureus* was statistically insignificant, whereas the difference between all other groups was statistically significant ($p < 0.05$).

Exposure to 7 kGy irradiation lead to a 5 log reduction of compared to the control group. The difference between all groups excluding groups irradiated at 1 and 2 kGy doses were found to be statistically significant ($p < 0.05$). The number of yeasts-molds in non-irradiated meatballs in the control group in our study was 6.70 log cfu/g, whereas exposure to highest irradiation dose of 7 kGy resulted in a reduction by 5.5 log, which lead to a number of 1.32 log cfu/g in yeasts and molds. The difference between all groups in terms of mold and yeast numbers is statistically significant ($p < 0.05$), and the difference maintained its importance at the end of 30 day storage. Hastings, Holzapfel, & Niemand (13) suggested that lactobacillus dominate in irradiated meat. Also, in our study lactic acid bacteria displayed a lower reduction and survived in the medium together with molds and yeast.

Meatballs examined in this study, following irradiation were stored in refrigerator, and analyzed on days 5, 10, 15, 20 and 30. The numbers of microorganisms were observed to increase regularly in each microorganism group during storage. An increase by 2.56 logs was observed in the control group in TAMB number at the end of 30 day storage.

The moisture content and average fat content of the meatballs were found to be 54.4% and 21.3%, respectively. The pH values, peroxide values (PV) and percent of free fatty acid (FFA %), which were measured throughout storage time in this study, were given in the fig. 8, 9 and 10.

Regarding of the pH values, it was found that lower doses of irradiation did not cause any significant changes in pH values, yet with high doses of irradiation higher pH values were determined for the products. The initial pH values for the meatballs were lower than the ones, which were reported by Yılmaz & Demirci (32) and İşgöz, Yıldızhan, & Özmumcu (14). This situation may be a consequence of the chemical properties and quantity of the additives such as sodium bicarbonate, added to the meatball batter. It was observed that in all groups pH values have increased during the storage, yet a faster increase was observed in the control group. The decrease in the pH values for the beginning of the storage period could be explained by the elimination of the Gram-negative bacteria and by the domination of the Gram-positive bacteria in the microflora. In the relatively higher dose irradiations, the smaller decrease in pH could be explained by the greater degree of elimination of Gram-positive bacteria at such doses. In the following days of storage the increase in the numbers of deteriorating microorganisms and deterioration occurring in time have caused an increase in pH values. The changes of pH values were very similar to the values that were reported by Lefebvre, Thibault, Charbonneau, & Piette (18).

The hydrolysis of triglycerides and phospholipids causing the production of free fatty acids has been reported by numerous researchers. In this study, it was found that irradiation had no significant effects on the FFA levels. Yet in all groups FFA levels increased with increasing lengthening of the storage. As the lipolytic deteriorating microorganisms are sensitive to irradiation, the increase in the FFA has been found to be lower in the irradiated samples. These results were similar to those reported by Dempster, Hawrysh, Shand, Lahola-Chomiak, & Corletto (8), Lefebvre, Thibault, Charbonneau, & Piette (18) and Kanatt, Paul, D'Souza, & Thomas (15). We also found that irradiation led to an increase in peroxide values, yet this increase was totally independent of the dose of irradiation. These results were very similar to those values that were reported by Groninger, Tappel, & Knapp (10), Dempster, Hawrysh, Shand, Lahola-Chomiak, & Corletto (8), Hanis, Jelen, Klir, Munukova, Perez, & Pesek (11) and Lefebvre, Thibault, Charbonneau, & Piette (18).

Conclusions

In this study, exposure to irradiation resulted in significant reduction in the number of bacteria in all meatball groups compared to non-irradiated (control). It was observed that with exposure to irradiation, the hygienic quality of the product was improved and reduction or total elimination would be envisaged in the number of pathogens, toxin producing and deteriorating bacteria which can create risk for human health. In the light of these study results, it is seen that intended objectives will be achieved with legally permitted doses of irradiation (4).

As a result of this study it was found that irradiation did not cause any significant changes in FFA levels, yet in all groups was seen increase in FFA levels with lengthening of storage. Irradiation was found to cause an increase in peroxide values, yet this increase was totally independent of the dosage of irradiation. When the irradiation is used in combination with a moderate heat treatment, addition of spices and vegetable extracts, the mediation dosage may be reduced to low levels without any effects on the product quality (17).

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