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Improvement of the Microbiological Safety of Two Chilled Semi-Prepared Meals by Gamma Irradiation**

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Summary

Experimental batches of a stuffed pasta product, tortellini, and slightly pre-fried breaded reconstituted turkey steaks with cheese and ham filling, Cordon Bleu, were prepared according to commercial recipes, then inoculated with 10^4 CFU/g of *Staphylococcus aureus* (in case of tortellini) and with 10^6 CFU/g of *Listeria monocytogenes* (in case of Cordon Bleu) prior to packing in plastic bags under a gas atmosphere of 20 % CO_2 and 80 % N_2. The inoculated packages were irradiated at 3 kGy (tortellini) and 2 kGy (Cordon Bleu) with a ^{60}Co radiation source. The applied radiation doses were sensorially acceptable for these products. The experimental batches of tortellini were stored at 15 °C, while the Cordon Bleu samples were stored at 5 and 9 °C. Unirradiated samples were kept together with the respective irradiated ones. Storage was continued for 4 weeks and microbiological tests were performed before and after the irradiation, and subsequently after every seven days. Besides selective estimation of the counts of the test organisms, total aerobic counts were evaluated in all samples and in case of Cordon Bleu, colony counts of lactic acid bacteria, Enterobacteriaceae, sulphite reducing clostridia, yeasts and moulds were also selectively estimated. The 3-kGy dose reduced the *S. aureus* count in tortellini below the detection limit (logCFU=0.26), and it remained undetectably low in the irradiated samples during all 28 days of storage, while the *S. aureus* count in the unirradiated samples increased up to 10^8 CFU/g during 8 days. The *Listeria* count in Cordon Bleu was reduced by irradiation from the initial count of 6.1 to 3.5 logCFU/g. At 5 °C storage, this residual count remained stagnant up to 3–4 weeks, but started to increase at 9 °C after one week of storage. In the unirradiated samples, the *Listeria* count increased hundred-fold during 4 weeks at 5 °C, and during 2 weeks at 9 °C. Sulphite reducing clostridia were, and remained, undetectable (<0.48 logCFU/g) in all samples even at 9 °C. The limiting factor of the shelf-life of the unirradiated poultry products was the growth of lactic acid bacteria at 9 °C, whereas enhanced lipid oxidation was an unwanted side-effect of radiation treatment. From these studies it can be concluded that the potential risk posed by the investigated non-sporeforming pathogenic bacteria could be considerably reduced by gamma irradiation, however, storage temperature remains a crucial factor of safety and methods should be developed to counteract the lipid-oxidative effect of the radiation processing.

Key words: gamma irradiation, tortellini, *Staphylococcus aureus*, Cordon Bleu, *Listeria monocytogenes*

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Introduction

On the market there is a growing interest in semi-prepared and prepared meals, packed in modified atmosphere (MAP) and distributed under chilled conditions instead of frozen. Such products are less energy-demanding and more attractive for the consumers than frozen meals. However, they are non-sterile and potential survival of some pathogenic microorganisms and/or post-processing contamination before packaging create microbiological risks, and a considerable limitation of shelf-life, especially under abusive temperature conditions which may frequently occur during retail display period, and home storage before consumption.

Irradiation is an effective way to eliminate non-sporeforming pathogens without changing the physical state of foods (*1*). Therefore, the objective of our work was to investigate the feasibility of diminishing the above risk by gamma irradiation of two types of chilled semi-prepared meals: a filled pasta product, tortellini, and slightly pre-fried reconstituted breaded turkey steaks containing cheese and ham filling (called Cordon Bleu). For challenge testings (*2*), experimental batches of tortellini and Cordon Bleu were inoculated with *Staphylococcus aureus* and *Listeria monocytogenes*, respectively. The test organisms were selected on the basis of their importance from the point of view of the product's microbiological safety; the radiation doses were chosen in view of preliminary experiences with sensorial effects on the studied products.

Materials and Methods

Test organisms and preparation of inocula

Staphylococcus aureus strain ATCC 6538, obtained with a code number of B01462 as a lyophilized culture from the National Collection of Agricultural and Industrial Microorganisms, Budapest, was used as inoculum for tortellini.

Resuscitation of the stock culture was made in brain heart infusion broth, BHI (Merck, 1.10493). Its growth was checked by a *Staphylococcus* enrichment broth and formation of typical colonies on Baird-Parker agar (Merck, 1.07809), as well as positive catalase and coagulase tests. Storage and subculturing were done on BHI and plate count agar slopes.

For preparation of the inoculum, the bacterium was cultivated with shaking in BHI broth at 37 °C for 24 h. The cells were harvested by refrigeration centrifugation for 15 min at 15 000 rpm, and resuspended in Sörensen's phosphate buffer at pH=7.0.

Listeria monocytogenes 4ab strain no. 10, obtained from the culture collection of Dr. B. Ralovich (National Meat Research Institute, Budapest), was used as test organism for Cordon Bleu. A 24-hour culture produced at 30 °C in BHI broth in a shaker bath was centrifuged as above for 15 min, and the sedimented cells were resuspended in sterile water.

Test materials

A commercially prepared tortellini pasta product, and Cordon Bleu containing bread crumbs and mildly pre-fried reconstituted turkey steaks filled with a slice of ham and a slice of cheese were used as test materials. Cordon Bleu is marketed presently only as an aerobically packed quick frozen food.

Tortellini dumplings were made from grits of durum wheat flour, egg and water, and their stuffing was prepared with bread crumbs, smoked ham, cheese, salt, pepper and Na-glutamate. The pasta/filling ratio was 25:9. Water activity of the product was approx. 0.96 at 25 °C, and the pH ranged between 5.5 and 6.1.

Water activity of Cordon Bleu was also approx. 0.96 at 25 °C, and the pH of the homogenized samples was in the range of 6.24–6.30.

Inoculation and packing of samples

Tortellini dumplings were inoculated individually to approx. 10^4 CFU/g level with 10-μL aliquots of the *Staphylococcus aureus* suspension prepared as above, and the inoculated pasta (10 dumplings per pouch) were packed by a Multivac Victus packaging machine with the packaging foil of the commercial pasta producer under a gas atmosphere of approx. 20 % CO_2 and approx. 80 % of N_2 (the initial O_2 content was 0.23 %).

Individual »steaks« of Cordon Bleu were inoculated with 120 μL of *Listeria monocytogenes* suspension, which was distributed in 10-μL aliquots on the surface of the lightly pre-fried steaks in order to obtain a *Listeria* contamination of approx. 10^6 CFU/g of the test material. The steaks were then vacuum-packed (10 bar residual pressure) into »Multibarrier-4« laminated foil bags which were then re-filled to 750 bar pressure with a gas mixture of approx. 20 % CO_2 and approx. 80 % N_2.

For both products twenty-five individual bags were inoculated.

Radiation processing

Half of the packages of the inoculated products were irradiated by an RHγ-30 type self-shielded ^{60}Co gamma irradiator at a dose rate of 2.4 kGy/h. Processing doses were 3 kGy for tortellini and 2 kGy for Cordon Bleu, sensorially acceptable doses selected by preliminary experiments reported elsewhere (*3*). Due to relatively short duration of irradiation, the temperature of the chilled products remained below the room temperature during irradiation. The unirradiated packages were kept together with the irradiated samples as controls.

Storage and microbiological testing

The experimental batches of tortellini were stored at 15 °C, representing a strongly abusive but not infrequent temperature condition, while Cordon Bleu samples were stored at 5 and 9 °C. Storage was continued for 4 weeks and microbiological testings were performed before and after the irradiation, and subsequently after every seven days of storage.

Besides selective estimation of the counts of the test organisms, total aerobic viable cell counts, and, in case of Cordon Bleu, also colony counts of lactic acid bacteria, Enterobactericeae, sulphite reducing clostridia as well as yeasts and moulds were selectively estimated from

appropriate dilution levels prepared from duplicate packages per treatment.

Estimation of *Staphylococcus aureus* was performed both by direct spread plating on Baird-Parker agar + tellurite egg yolk emulsion (Merck, 1.03785) at 37 °C incubation for 48 h, and by *Staphylococcus* enrichment broth tubes (Merck, 1.07899), with K_2TeO_3 (Merck, 1.05164). Selective estimation of *Listeria monocytogenes* was done both in Oxford agar (Merck, 1.07004) with *Listeria* supplement (Merck, 1.07006), which was counted after 24 and 48 h at 30 °C, and also by MPN technique, in triplicate liquid cultures using *Listeria* enrichment broth (Merck, 1.10259) and incubation at 30 °C for up to 5 days. *Listeria* positive tubes were verified by subculturing them onto Oxford agar plates.

Total aerobic plate counts were estimated in PCA (casein-pepton-dextrose-yeast agar, Merck, 1.05463) at 30 °C incubation for 2–3 days. The counts of bacterial spores were determined by plating as the total aerobic counts after 10 min of heat treatment at 80 °C. Lactic acid bacteria were counted by plating into MRS medium (Merck 1.10661) with 1.5 % agar, covered after inoculation with a surface layer of the same medium, and incubated at 30 °C for 3 days. Enterobacteriaceae counts were estimated by plating in VRBG agar (Oxoid, CM485), overlayered with the same medium, and incubated at 37 °C for 24 hours. Sulphite reducing clostridia were investigated by plating into differential clostridial agar (DCA) according to Wenk (Merck, 1.10259) supplemented with ferric-ammonium-citrate (1 g/L) and Na_2SO_3 (0.75 g/L), incubated in Oxoid HP11 anaerobic jars containing Oxoid Anaerogen AN35 oxygen adsorbent. Yeasts and moulds were estimated in thin layers of DRBC agar (Oxoid CM 727) with chloramphenicol and supplement (Oxoid SR 078E), incubated for 6 days at room temperature.

In each experiment two independent packages were tested separately for their microbiological status and mean values of log colony counts from triplicate plates of relevant dilution levels are given in the tables as individual figures.

Measurement of the head-space gas-composition

The head-space of the MAP samples was checked by a Servomex IR Gas Analyser PA 404 equipment for CO_2, and by a Servomex Oxygen Analyser 574 for O_2 content.

Measurement of pH and water activity

The pH was measured by a Physitemp electric pH meter and a_w was estimated by the crystal liquefaction method (*4*).

Thiamine content

Thiamine (vitamin B_1) content was estimated by a microbiological method (*5*).

Lipid oxidation

Lipid oxidation was investigated by determination of thiobarbituric acid reactive substances (TBARS values) according to Newburg and Concon (*6*), expressed in malonaldehyde (MA) concentrations. Malonaldehyde is an oxidative breakdown product formed mainly from peroxidized polyunsaturated fatty acids.

Results

Radiation processing of MAP-chilled pasta product tortellini

Microbiological effects

Results of the microbiological tests in the preliminary studies with tortellini are summarized in Table 1.

The initial total aerobic viable cell counts of the inoculated samples were determined by the *S. aureus* inoculum, because the original total aerobic cell count of the unirradiated samples was 2 log cycles shorter, approx. logCFU/g=2.3, composed mainly of aerobic bacterial spores.

As an effect of irradiation, the *S. aureus* count was reduced below the detection limit (which was lower in case of the MPN technique), *i.e.* less than logCFU=0.26. Thus, the radiation treatment resulted in more than a 3 log cycles' reduction of the test organism in the pasta product.

Considering the initial total aerobic counts of the irradiated samples at the beginning of the storage (log CFU/g≈1.5), the aerobic spores of the »native« microbiota were reduced by somewhat less than one log cycle by the 3 kGy dose. The surviving sporeformers were unable to grow in the irradiated samples under the experimental conditions.

The *Staphylococcus aureus* inoculum grew up to 10^8 CFU/g level in the unirradiated samples within 8 days,

Table 1. Logarithm of viable cell counts in duplicate samples of tortellini stored at 15 °C

Viable cell counts	Storage time/day							
	0		8		14		28	
	0 kGy	3 kGy	0 kGy	3 kGy	0 kGy	3 kGy	0 kGy	3 kGy
Total counts	4.32	1.48	7.99	4.52	8.11	4.81	ni	5.75
	4.28	1.54	7.91	4.18	8.04	4.70	ni	7.43
S. aureus (Baird-Parker)	4.08	<1.70	7.98	<1.70	8.04	<1.70	ni	<1.70
	4.04	<1.70	7.95	<1.70	8.04	<1.70	ni	<1.70
S. aureus (enrichment broth)	4.08	<0.26	7.88	<0.26	7.36	<0.26	ni	<0.26
	4.67	<0.26	8.36	<0.26	7.36	<0.26	ni	<0.26

ni = not investigated

however, no *S. aureus* cells were found in the irradiated batch during the 28 days of storage at 15 °C.

The aerobic total viable cell counts started to increase in the irradiated samples only after 8 days of storage, and their slow increase afterwards was due to some yeasts and micrococci forming white colonies distinctly different from those of the test organism.

Thiamine content

The thiamine content of the untreated samples was found to be 34 μg/100 g, with an accuracy of ±10 %, and it was not changed by the radiation dose applied.

Radiation processing of the MAP-chilled poultry product Cordon Bleu

Microbiological effects

In order to see how many microbial loads were carried by the pre-fried Cordon Bleu, individual components of uninoculated and unirradiated steaks were investigated for aerobic total counts, bacterial spores (cells surviving 80 °C for 10 min) and viable cell counts of lactobacilli, at the beginning of the storage experiment. The results are shown in Table 2.

These results illustrate that, although in relatively low number, not only bacterial spores but also vegetative bacterial cells could be recovered from each component of the product. Therefore, in principle, there is an opportunity that eventual pathogenic contamination might occur in such a product.

The *Listeria* counts and the counts of lactobacilli of the inoculated samples are shown together with the pH values in Tables 3 and 4 as a function of irradiation and the storage time. Due to the heavy inoculation, and the low number of the »native« microbiota, the total viable cell counts are not shown, because they were equal to the *Listeria* counts. Other types of selectively investigated microorganisms (spore counts, Enterobacteriaceae, yeasts and moulds) were and remained under the detection level of logCFU/g=0.48.

Table 2. Microbial load of individual components of the uninoculated and unirradited Cordon Bleu (logCFU/g values of duplicate samples)

Component	Total aerobic count	Spore count	Lactobacilli
Crumb	2.30	0.95	<0.48
	1.84	0.78	<0.48
Turkey meat	2.15	0.48	1.48
	2.73	0.48	1.89
Ham	1.32	1.48	<0.48
	1.71	<0.48	<0.48
Cheese	2.36	1.89	0.78
	2.32	1.82	0.78

The *Listeria* count in Cordon Bleu was reduced by irradiation from the initial logCFU/g=6.1 to 3.5. At 5 °C storage (Table 3), this residual count remained stagnant for up to 3–4 weeks, but at 9 °C (Table 4) it started to increase after the first week. In the unirradiated samples, the *Listeria* count increased hundred-fold during 4 weeks

Table 3. Microbiological changes of inoculated unirradiated and inoculated irradiated (2 kGy) MAP Cordon Bleu at 5 °C storage temperature (logCFU/g values of duplicate samples)

Parameter	Untreated					Irradiated				
	t/day					*t*/day				
	1	7	14	21	28	1	7	14	21	28
Listeria counts	6.08	6.18	6.73	7.48	8.08	3.53	3.30	2.92	2.62	5.15
	6.15	6.15	6.66	7.28	7.56	3.57	3.26	2.89	2.68	3.52
Lactobacilli	<0.48	<0.48	1.26	4.00	6.41	<0.48	<0.48	<0.48	<0.48	<0.48
	<0.48	<0.48	<0.48	6.11	6.23	<0.48	<0.48	<0.48	<0.48	<0.48
pH	6.17	6.14	6.02	6.02	5.73	6.15	6.14	6.03	6.08	6.03
	6.16	6.12	6.04	6.00	5.87	6.14	6.13	6.04	6.07	6.02

Table 4. Microbiological changes of inoculated unirradiated and inoculated irradiated (2 kGy) MAP Cordon Bleu at 9 °C storage temperature (logCFU/g values of duplicate samples)

Parameter	Untreated					Irradiated				
	t/day					*t*/day				
	1	7	14	21	28	1	7	14	21	28
Listeria counts	6.08	6.98	8.15	8.90	8.51	3.53	3.74	6.52	7.71	8.11
	6.15	7.43	8.04	8.46	8.41	3.57	3.46	6.46	7.79	8.15
Lactobacilli	<0.48	<0.48	1.62	4.15	7.41	<0.48	<0.48	<0.48	<0.48	<0.48
	<0.48	<0.48	<0.48	6.36	8.04	<0.48	<0.48	<0.48	<0.48	<0.48
pH	6.17	6.07	5.74	5.74	5.56	6.15	6.13	6.04	5.94	5.72
	6.16	6.06	5.72	5.63	5.58	6.14	6.14	6.05	5.92	5.75

Table 5. The volume fractions of CO_2 and O_2 in the head-space of MAP Cordon Bleu as a function of storage time and temperature (measurements of duplicate packages)

Treatment	T/°C	$\varphi(CO_2)$/ %						$\varphi(O_2)$/%					
		t/day						t/day					
		Initial	1	7	14	21	28	Initial	1	7	14	21	28
0 kGy	5	19	10	12	10	12	12	1.4	2.0	2.0	1.6	1.8	1.9
				12	11	8	12			1.8	1.5	1.8	1.8
	9	20	12	14	10	20	18	1.4	1.8	1.7	1.5	1.7	1.7
				12	11	17	18			1.4	1.4	1.7	1.7
2 kGy	5	20	11	9	11	10	10	1.5	1.7	1.8	1.3	2.0	2.0
				12	12	11	11			1.7	1.0	2.0	2.0
	9	18	11	12	15	14	16	1.4	1.7	1.3	0.8	1.9	2.0
				10	12	13	14			1.7	0.4	1.9	1.9

at 5 °C, and during the first 2 weeks at 9 °C. Limiting factor of the microbiological shelf-life of the unirradiated product was the growth of lactic acid bacteria at 9 °C, although they were in undetectably low number during the first two weeks of storage. They apparently survived the pre-frying process heterogeneously distributed and in very low counts inside the product.

The radiation treatment was effective in eliminating this low number of lactic acid bacteria. In spite of the buffer capacity of the samples, the decrease of the pH in the unirradiated samples reflected duly the growth of lactic acid bacteria. However, a slight decrease of the pH was observed also in the irradiated samples during the course of storage, when an extensive growth of the surviving *Listeria* was observed.

Gas composition of the head-space of the experimental product

The volume fractions of CO_2 and O_2 in the head-space of the packages as a function of the storage time and temperature are given in Table 5.

These measurements revealed that considerable part of the CO_2 introduced during packaging was soon dissolved in the high-moisture product. Thus, the equilibrium CO_2 volume fraction in the head-space was 10–12 % in the packages stored at 5 °C, while at 9 °C the head-space volume fraction of CO_2 increased again when the bacterial growth became intense. The O_2 volume fraction was most frequently between 1.5 and 2.0 %, irrespective of the storage temperature and time.

Appearance of the samples and lipid oxidation

The radiation treatment did not change the appearance of the samples and no off-odour was detected at the opening of the bags in the first half of the storage period. However, after 2 weeks of the refrigerated storage, the bread-crumb coat of the steaks started to develop a less freshly-fried, more moist appearance, independent of the radiation treatment. Cheese slice inside the unirradiated samples started to liquefy more intensely at 9 than at 5 °C. During the last two weeks of storage the samples were loosing gradually their freshness, attractive appearance and produced some oily exudation. The untreated samples developed a »stale« odour, while in the irradiated samples a rancid odour was noticed when they were prepared with a homogenizer for microbiological testing.

TBARS values of Cordon Bleu samples directly after irradiation and 4 weeks of storage are given in Table 6. The increased TBARS values showed that lipid oxidation progressed during storage and it was enhanced by the radiation treatment.

Table 6. TBARS values of Cordon Bleu samples as a function of irradiation and storage

Radiation dose / kGy	w(TBARS)/(mg/kg) in MA					
	Directly after irradiation		After 4 weeks of storage at			
			t=5 °C		t=9 °C	
	mean	S.D.	mean	S.D.	mean	S.D.
0	0.38	0.04	0.85	0.03	1.00	0.24
2	0.51	0.03	1.58	0.20	1.86	0.25

Discussion

International literature about filled pasta products shows that the incidence of contamination with *Staphylococcus aureus* is not infrequent (*7–11*). The water activity level of the product investigated in our experiments would not exclude either the opportunity of growth or enterotoxin formation of *S. aureus* if the pathogen contaminated this type of product. Because of the high heat resistance of *Staphylococcus* enterotoxins, the final cooking of this type of stuffed pasta cannot inactivate the preformed toxins. Therefore, we performed the inoculated pack studies described. Our experiments showed that *Staphylococcus aureus* was growing readily at 15 °C in the inoculated tortellini of a_w=0.96, but the 10^4/g of artificial contamination level of the pathogen could be eliminated from the experimental samples by 3 kGy gamma irradiation, a sensorially acceptable dose level, which did not decrease the thiamine content of the product.

The present commercial production technology cannot fully assure the safety of the poultry product Cordon Bleu for non-frozen storage even under modified atmosphere packaging, because even some vegetative

bacteria might survive the mild pre-frying of the product. Survival and growth of lactic acid bacteria in the unirradiated experimental batches make it probable that *Listeria monocytogenes*, an ubiquitous environmental contaminant, might eventually be present, too, and it is able to multiply during refrigerated storage. *Listeria* count in Cordon Bleu was reduced by 2.6 log units using the sensorially acceptable 2 kGy gamma radiation dose. This extent of lethality is close to the result obtained by Thayer *et al.* (*12*) who found that the D value of *Listeria monocytogenes* was (0.63±0.06) kGy in cooked ground turkey meat. The observed reduction of *Listeria monocytogenes*, the most radiation resistant non-sporeformer (*13*), could result in a *Listeria*-free product, if the contamination level of this pathogen was not higher than 10^2 CFU/g. In our heavily contaminated experimental batch, the surviving level of the test organism remained stagnant at 5 °C for up to 3–4 weeks in the irradiated samples, while the *Listeria* counts of the unirradiated samples increased hundred-fold during 4 weeks at 5 °C, and during 2 weeks at 9 °C. In irradiated samples stored at 9 °C, the surviving *Listeria* also started growing after one week. This slow recovery of the radiation survivors is in agreement with the observation of Patterson *et al.* (*14*) who irradiated raw and cooked poultry meat inoculated with *L. monocytogenes* to 0, 1.0 and 2.5 kGy and found that irradiation resulted in significantly increased lag times for this pathogen. Regarding psychrotrophic sporeforming pathogens, it is reassuring that sulphite-reducing clostridia were and remained undetectable during the entire period of our experiments, even in the unirradiated samples. Our results can be compared with those of Hashim *et al.* (*15*) who irradiated chicken meat to 1.66 and 2.86 kGy, and determined the effects of radiation processing on the sensory attributes of both raw and cooked meat, and did not find significant effects upon the appearance, or taste of cooked breast meat. Kanatt *et al.* (*16*) irradiated minced chicken to 2.5 kGy and stored it at 0 to 3 °C for up to 4 weeks. The irradiated meat was microbiologically safe and sensorially acceptable in the unfrozen state up to the end of this storage; the unirradiated minced chicken had a shelf-life of less than 2 weeks.

Conclusions

One can conclude from these studies that the potential microbiological risk in the experimental products posed by the investigated non-sporeforming pathogenic bacteria could be considerably reduced by sensorially acceptable radiation doses. However, storage temperature remains a crucial factor of safety, and increased lipid oxidation is a limiting factor of shelf-life of the irradiated poultry product even under low O_2 volume fraction of the MAP. The possibility of the counteraction of lipid oxidation by efficiently antioxidative additives is the aim of our further studies.

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Poboljšanje mikrobiološke sigurnosti dvaju hlađenih polupripravljenih jela gama-zračenjem

Sažetak

Eksperimentalni uzorci punjenih tortelina i prethodno prženih paniranih purećih odrezaka punjenih sirom i šunkom (Cordon Bleu) pripravljeni su prema komercijalnim receptima te inokulirani s 10^4 CFU/g bakterije *Staphylococcus aureus* (u uzorcima tortelina) odnosno s 10^6 CFU/g bakterije *Listeria monocytogenes* (u uzorcima Cordon Bleu) prije umetanja u plastične vrećice pod atmosferom od 20 % CO_2 i 80 % N_2. Vrećice su bile ozračene s ^{60}Co od 3 kGy (tortelini) i 2 kGy (Cordon Bleu). Primijenjene radijacijske doze bile su senzorski prihvatljive. Eksperimentalni uzorci tortelina bili su uskladišteni pri 15 °C, a uzorci Cordon Bleu pri 5 odnosno 9 °C. Neozračeni uzorci čuvani su zajedno s ozračenima. Skladišteni su 4 tjedna, a mikrobiološko testiranje provedeno je prije i nakon zračenja, te nakon svakih 7 dana. Osim selektivne procjene broja test-organizama, u svim je uzorcima procijenjena količina ukupnih aeroba, a u uzorcima Cordon Bleu utvrđena je količina kolonija bakterija mliječne kiseline, *Enterobacteriaceae*, sulfitoreducirajućih klostridija, kvasaca i plijesni. Zračenje od 3 kGy smanjilo je u tortelinima broj stanica bakterije *S. aureus* ispod granice detekcije (logCFU=0,26), što je ostalo nepromijenjeno tijekom svih 28 dana skladištenja. Broj stanica bakterije *S. aureus* u neozračenim uzorcima povećao se do 10^8 CFU/g tijekom prvih 8 dana. U Cordon Bleu zračenjem se smanjio broj stanica bakterije *Listeria*, od početnih 6,1 na 3,5 logCFU/g. Pri temperaturi skladištenja od 5 °C taj se broj zadržao 3–4 tjedna, a počeo se povećavati pri temperaturi od 9 °C nakon jednog tjedna. U neozračenim uzorcima broj stanica bakterije *Listeria* povećao se 100 puta tijekom 4 tjedna pri 5 °C, a pri 9 °C već tijekom dva tjedna. Sulfitoreducirajuće klostridije nije bilo moguće utvrditi (<0,48 logCFU/g) ni u jednom uzorku, pa ni pri 9 °C. Limitirajući faktor trajnosti neozračenih purećih odrezaka bio je rast bakterija mliječne kiseline pri 9 °C, dok je pojačana oksidacija lipida bila neželjena nuspojava pri obradi zračenjem. Ta su istraživanja pokazala da se gama-zračenjem znatno smanjuje opasnost od patogenih bakterija koje ne stvaraju spore, a pritom je temperatura skladištenja bitan faktor. Trebalo bi pronaći postupke koji bi spriječili oksidaciju lipida kao posljedicu zračenja.