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IMPROVING THE QUALITY OF MEAT PRODUCTS THROUGH GAMMA IRRADIATION

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ABSTRACT

The aim of the current study is to determine the effect upon experimental meat product samples (flat sausage) prepared with start bacterial cultures, sterilized through irradiation under their biological effectiveness. The experimental types of meat were divided into 3 groups: flat sausage with Megacarn starters, flat sausage with Lactina starters and flat sausage without starters. These groups have been irradiated with 4kGy gamma rays. Biological experiment with white male mice weighted 20 - 30 g, took place. The experimental species were separated into groups of ten and were fed on the studied types of sausages for a period of 20 days. On the 20th day the species were irradiated with 7.5Gy. Mice separately irradiated in advance with 3.5Gy were fed on flat sausage with Megacarn starters. On the 20th day this group was once again irradiated with 7.5Gy. Studied were their weight, survival and the number of leucocytes. Our results showed that after the first irradiation, 40% of the animals fed on non irradiated flat sausage with Megacarn starters died and after the second irradiation 60% of them died. In the group fed on Lactina starters, 100% from the non irradiated and 80% from the irradiated species died. In our study we established that leucocytes restoration was delayed in the mice from the group fed on non irradiated samples. On the 20th day feeding the irradiated animals had a 60% death rate in the group fed on irradiated Megacarn, 10-20% death rate in the group fed on irradiated Lactina and 0% in the control group fed on non irradiated food. The presence of staphylococcus in Megacarn starters gave us grounds to test it independently. The experimental scheme was repeated but the animals were fed on Megacarn water solution. The experimental groups of animals were irradiated with 4kGy and after 20 days on Megacarn water solution they were once again irradiated with 8Gy. During the first irradiation we did not observe dynamical changes in the studied criteria. After the second irradiation the control group resulted in 60% death rate and the group fed on Megacarn water solution resulted in 30-40% death rate.

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INTRODUCTION

Finding proper methods for food processing aiming at preserving their nutritional characteristics and extending their shelf life is of greatest importance. After the traditional methods of processing, recently a new opportunity has appeared – processing by means of ionizing radiation. Irradiation of foods is identified as a risk-free technology for reduction of food spoiling, thus becoming an active part in the production of high quality products, their processing and preparation. Food safety is an issue of increasing importance to consumers. Scientists, regulatory authorities and a number of legal organizations work to determine the best way to fight diseases caused by contaminated foods and encourage the use of technologies that can ensure safety for the national food stock. More than 50 years of research has gone into our understanding of the safe and effective operation of irradiation as a food safety measure-more than any other technology used in industry today. Food irradiation employs controlled amounts of ionizing (having sufficient energy to create positive and negative charges) radiation to destroy bacteria, pathogens, and pests in food and agricultural products, greatly reducing the threat of foodborne disease. Many experts estimate that irradiating half of all ground beef, poultry, pork and processed meat would reduce food poisoning by one million cases and prevent 6,000 serious illnesses and 350 deaths (Tauxe, 2001). Ionizing radiation includes gamma rays (from radioactive isotopes cobalt-60 or cesium-137), beta rays generated by electron beam or "E-beam", and X-rays. None of these

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irradiation sources has sufficient energy to be capable of inducing radioactivity; however, they do have enough energy to remove electrons from atoms to form ions or free radicals. The amount of ionizing radiation absorbed is termed radiation absorbed dose and is measured in units of grays (1Gy=100rad), with 1Gy equal to 1 Joule/kg and 1,000 Gy equal to 1 kiloGray (kGy). Gamma rays and X-rays are able to penetrate further into foods than beta rays. During irradiation the food is exposed for short time to a radiation source in a measuring device environment. Irradiation does not substitute manufacturing and manual procedures in the course of manufacture of food products, but the process, when used for treatment of meat and poultry, may eradiate disease bearing bacteria and highly reduce potential risks (WHO, 1981). Several extensive reviews of toxicological and other data by regulatory and health organizations, including Health Canada (2003), FDA (1986), Codex Alimentarius Commission (CAC,1983), and European Commission's Scientific Committee on Food (EC,2003), have determined that food irradiated below 10kGy is safe More recently, the CAC(2003) revised slightly its General Standard for Irradiated Foods, stating that the maximum absorbed dose delivered to a food should not exceed 10kGy, except when necessary to achieve a legitimate technological purpose.

In 1999, a joint study group of U.N.'S Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA), and World Health Organization (WHO) concluded that food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate. The group also concluded that no upper limit on absorbed dose was necessary because use of irradiation would be limited to doses that do not detrimentally impact the sensory attributes, thus creating practical cut-off at about 50-75 kGy (WHO, 1999) The group's report included all pertinent animal feeding studies (82 in total), mutagenicity studies (47 in vitro), and food type and test species through 1997. Although 14 studies showed an effect, the cause was attributed in each case to a diet/nutrient deficiency, not irradiation. It is important to remember that these trials involved feeding diets containing significant amounts of food items (average 35%-100%) irradiated at very high doses, often to 59kGy. There were eight possible effects of high dose radiation observed in the mutagenicity studies. Two of the studies involved feeding irradiated oils, which apparently caused extensive oxidation and loss of carotenoids. The other six studies used irradiated simple sugar solutions (e.g., sucrose, fructose, glucose, etc.) that are now known to involve formation of mutagens by radiation-induced chemical mechanisms (Fan, 2003).

In 1976, Scientific research group in USA carry out comprehensive nutritional, genetic, and toxicological studies of food irradiation. Mice, hamsters, rats, and rabbits were fed chicken (as 35-70% of their diet) that had been irradiated at a minimum absorbed dose of 46 kGy. Dogs, rats, and mice were also fed the irradiated chicken at 35% of their diet during multigenerational studies. They found no evidence of genetic toxicity or teratogenic effects in mice, hamsters, rats, or rabbits and no treatment-related abnormalities or changes in the multigenerational studies (Thayer et al., 1987). The problem of safety is considered from three aspects – mutagenic, toxicological, and from the viewpoint of changing the nutritional and sensory characteristics of food products (CAC, 1983). Tests on different species of animals have been carried out – mice, rats, dogs, monkeys, as well as people volunteers, showing no toxic effects from consuming different types of gamma irradiated food products, both for consumers and their descendents. In many countries such as the USA, Canada, Israel, Japan, etc. food irradiation is officially permitted and is supervised by the relevant sanitary-hygiene authorities. Food irradiation in doses up to 10κGy is permitted as there is no risk for their safety and no nutritional problems occur (Diehl J.F., 1991, 1995). Consumers need broader information in order to assess the advantages and safety of radiation technology, because in many places in the world there are organizations and groups of individuals which radically deny and do not accept this type of food processing (Kwon Joong-Ho, et al 1992). The major issue in relation to consumption of radiation treated foods is whether they are risk-free because radiolitic products and free radicals are formed in them. However, these are products formed during a more trivial food processing, such as: heating, toasting bread in toasters, etc. long-term laboratory experiments with animals fed on foods irradiated with up to 45κGy did not reveal any deviations from their usual life functions and no tumor formations has been established (Diehl J.F., 1995, Merritt C.1989).

Food products irradiation is used in pasteurization, stimulation of vegetable sprouting, delay of fruit ripening, fight against parasites and pests, as well as for extending shelf life of cooled meat, meat products, herbs, etc. the use of ionizing rays reduces the addition of nitrates in meat products manufacturing. The scientific study in this field is focused on answering questions about inclusion of irradiation combinations for foods with other well-known technologies and food processing. In the future efforts of the involved regulatory organizations should be directed to dissemination of information in public space about the advantages of the food irradiation technology. The final result will be an enormous public support, increase of consumer's safety and public acceptance of irradiated foods.

MATERIALS AND METHODS

In order to achieve the set objective raw-dried sausages with two types of biostarters were produced, prepared according to the standard requirements of the Bulgarian State Standard /BDS 2589-83/. In the day of production part of the meat sausages were irradiated with 4kGy dose with the gamma-irradiation installation *Gamma-1300* with radiation source ^{137}Cs and dose power of 1.75Gy/min. For faster 'ripening' of the product part of the experimental groups were inoculated with bacterial starter cultures. We used two types of starter cultures - biostarter*Lactina*1: *St. carnosus*, *St. xylosus*, *Pediococcuspentosaceus* + glucose and biostarter*Megacarn*2: *Lbs. plantarum*, Micrococcus variants + glucose. /1/ Samples were distributed as follows: first group – non-irradiated without a biostarter; second group – irradiated with 4kGy, without a biostarter; third group – non-irradiated with biostarter 1; fourth group – irradiated with 4kGy with biostarter 1; fifth group – non-irradiated with biostarter 2; sixth group – irradiated with 4kGy with biostarter 2; The physicochemical characteristics of the samples were determined according to standard methods. Lipids auto-oxidation processes were studied by determining the following indicators: determining secondary products of oxidation expressed through the quality of malonedialdehyde /MDA/ and thiobarbiturate number according to Newburg and Concon's method /1/. The microbiological studies of the sixth lots of

meat were carried out in compliance with BDS 6835-74 requirements. All of the samples were subject to sensory analysis by means of 9 points marking hedonic scale. The evaluators were a constant group of 10 tasters. The results of these analyses were reported at the XXXIV annual meeting, 2004 – Experimental models study of meat products, prepared with start bacterial cultures, sterilized through irradiation. The biologic experiment was carried out with experimental animals – male white mice weighing 25-30 g. The mice were divided in groups of ten and were fed for 20 days with the irradiated and non-irradiated types of meat product – first to sixth group (stated above). On the 20th day the mice were irradiated with 7.5 Gy gamma rays. For the purposes of the biologic experiment we used as food only Biostarter 2 which we irradiated with 4kGy. We fed mice irradiated in advance with a doze of 3.5 Gy gamma rays. The samples were divided as follows: seventh group – control group; eighth group – fed on Biostarter 2 (non-irradiated); ninth group – fed on Biostarter 2 (irradiated). Weight, survival rate and leucocytes changes were tested. We performed DNA extraction PFGE, SSCP, RAPD. The gels were photographed and analysed with the software package GelCompar, version 4.0 (Applied Maths, Kortrjik, Belgium).

RESULTS AND DISCUSSION

In order to determine the biologic efficiency of meat products manufactured with two types of start cultures: 1 – *Lactina* and 2 – *Megacarn*, and then irradiated with gamma rays we carried out a biological experiment. In the first series the experimental animals were divided into 6 groups and were fed on raw-dried sausage for 20 days. On the 20th day the sixth group of mice was irradiated with 7.5 Gy. The second series of experiments involved 3 groups of animals irradiated in advance with 3 Gy – control group № 7, the animals were fed on their usual food, second group № 8 – the animals were watered with dissolved starter 2, and third group № 9 – with dissolved irradiated starter 2. The animals were fed for 20 days. On the 20th day 3 groups of mice were irradiated with 7.5 Gy.

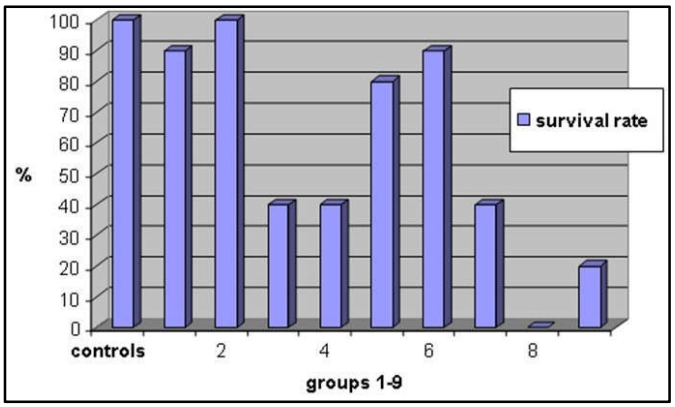

Figure 1. Survival rate of irradiated mice with dose 3.5Gy and fed with meat products treated withstarters for 20 days

Results revealed (Fig.1) that in group 1 and 2 there was a slight difference in the survival rate – 90% and 100%. All animals in group 3 survived while the animals in group 4 fed on irradiated product treated with starter 1, 40% survived; for groups 5 and 6 we had the following situation: mice of group 5 that were fed on untreated meat product with starter 2 achieved 80% survival rate in contrast to group 7 which had a 90% survival rate.

The leucocytes in these groups showed that restoration was delayed in the group fed on meat products with starter 1 for group 4, starter 2 for group 5; achieving the initial values was observed in 1 and 2 control groups and group 6 which was fed on meat product treated with starter 2 and irradiated with dose of 4kGy. Results revealed that after the first irradiation 40% of the animals fed on non-irradiated meat product with starter 2 died, and after the second irradiation – 60% of the animals died. We observed the delay in the restoration of leucocytes in the group with non-irradiated sample. For animals radiated, on the 20th day, after feeding we established a 60% death rate in the groups radiated and irradiated with starter 2, 0% in the control group and 10-20% death rate in the other two types.
Fig. 2 shows that the restoration of leucocytes in the three groups was delayed and did not achieve the initial values.

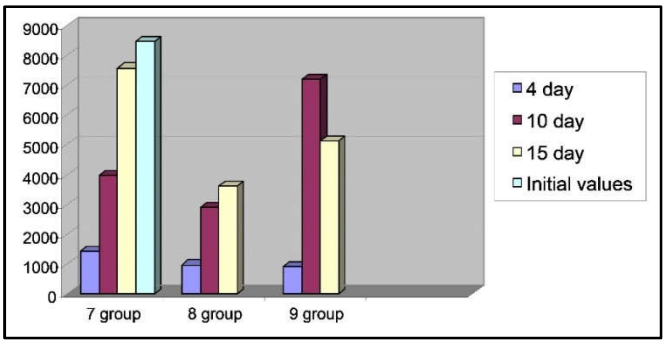

Figure 2. Changes of peripheral leucocytes in different terms after feeding with meat products

The sub-lethal irradiation with 3 Gy led to a 60% survival rate in 8th group–non-irradiated flat sausage Megacarn, while we had a 100% survival rate in the groups 7 and 9, as showed on figure 3.

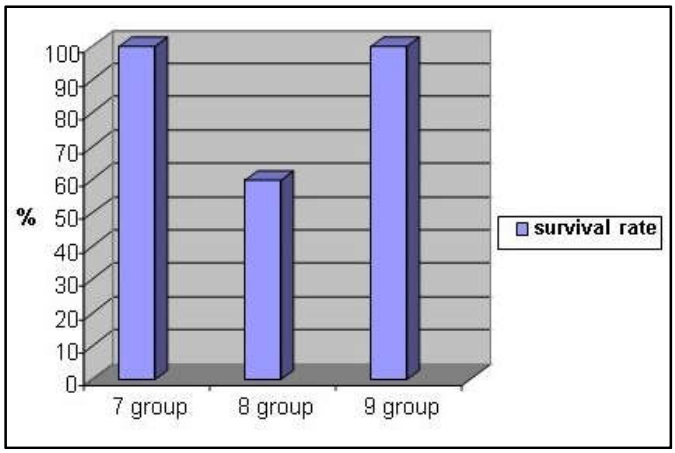

Figure 3. Survival rate of irradiated mice with dose 7.5Gy and fed with meat products treated with starters for 20 days / groups 7,8,9 after the first irradiation and feeding with raw-dried sausages with starter 2 /

The first series of experiments showed that with sub-lethal irradiation with 3.5Gy the survival rate did not reach 100% in the control groups in comparison to the animals fed on irradiated meat products. The leucocytes in these groups showed that the restoration was delayed. We observed 100% and 90% survival rate in animals from experimental groups 2 and 6, respectively. The presence of staphylococci in start culture 2 made us test it independently with irradiation in the course of the second series of experiments. The previous

scenario was repeated - the animals took the culture dissolved in drinking water, and not inoculated in a raw-dried meat product. The three groups /7, 8 and 9/ were irradiated in advance with 3Gy and after 20 day watering were irradiated for a second time with 7.5Gy. During the first irradiation we observed some differences in the survival rate and the leucocytes dynamics. For the groups irradiated on the 20th day of watering with 7.5Gy, the death rate was 60% in control groups and 30-40% in animals watered with starter 2. During irradiation with 7.5 Gy the lowest survival rate is characterized for groups 8 and 9 with starter 2 /fig. 3/. In order to check the importance of the bacterial start culture for the observed higher death rate of animals fed on meat product with starter 2, we made an experiment with 6 groups of mice. The mice were watered with dissolved starter for 20 days. Three of the groups were irradiated with 3.5Gy in advance. We observed some differences in the survival rate and leucocytes restoration in the animals irradiated with 3.5 Gy, Fig. 4, 5, 6.

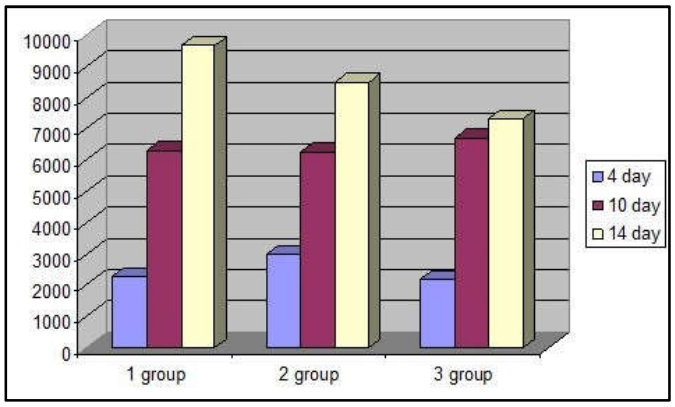

Figure 4. Changes of peripheral leucocytes in different terms after feeding with meat products

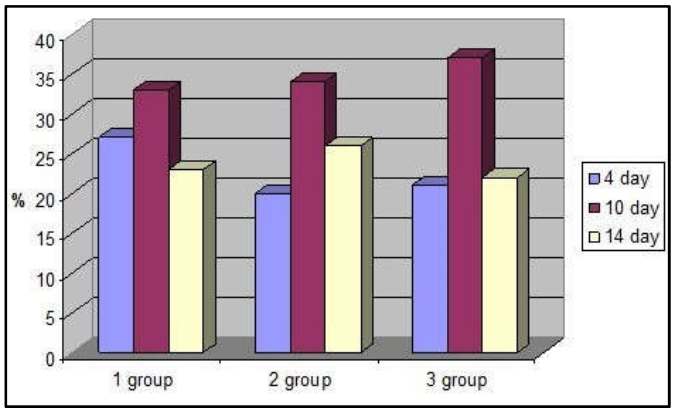

Figure 5. Changes of nuclear part leucocytes in different terms after feeding with meat products

On the 20th day of watering all animals were irradiated with 7.5 Gy. The survival rate of animals irradiated twice was 10-20%, and in groups with a single irradiation, the control group had a 40% survival rate, and the watered irradiated and non-irradiated groups – 70-60% respectively /Fig.7/. The results of the experiments showed that the higher death rate and the worse blood indicators of mice fed on samples with biostarter 2 – *Megacarn*, are not due to the bacterial factor, but most probably to some processes during the fermentation of flat sausages manufactured with such a start culture. In order to check the effect of irradiation on the strains of starters used for increasing the rate of ripening of the meat product, and to eliminate the eventual mutagenic effect of the used dose of 4 kGy on microorganisms, we carried out series of molecular analyses, the detailed results of which will be shown another publication.

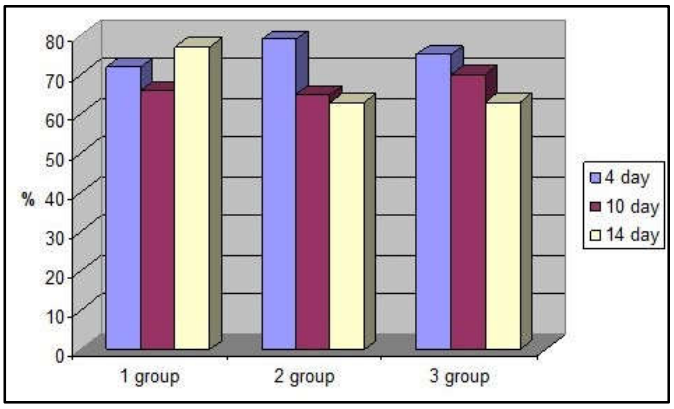

Figure 6. Changes of peripheral leucocytes in different terms after feeding with meat products

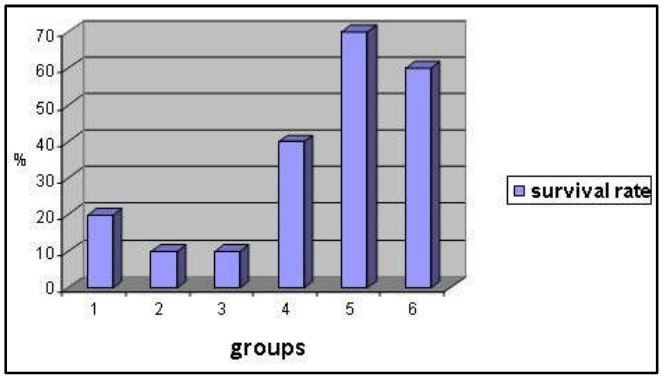

Figure 7. Survival rate of irradiated mice with dose 7.5Gy and fed with meat products treated with starters for 20 days / groups1,2,3 after the first irradiation and feeding with raw-dried sausages with starter 2

The pulse electrophoresis and RAPD are performed in order to confirm whether the different isolates of certain bacterial species are actually the same strains. They roughly determine whether there are some significant changes in the genome before and after radiation treatment. With SSCP analysis, however, the electrophoretic motility of fragments depends not only on the molecular mass, as the case is with pulse electrophoresis and RAPD, but also on the sequence. That is to say SSCP may detect mutations – even spot mutations. And provided the profiles prior irradiation coincide with these after irradiation, we can state that for the selected random fragments there are no changes in the DNA sequence. Determining the effect of a certain factor, in this case – of irradiation, on the entire genome is performed with the so called re-sequencing with DNA *microarrays*. I.e., first, a sequencing of the entire genome is made, using a capillary-electrophoresis sequencer (which requires at least three months, and there is no such possibility in Bulgaria), and then after irradiation the bacterial DNA is subjected to re-sequencing using DNA *microarray* last generation chips (this is impossible not only in Bulgaria, but anywhere in Eastern Europe). This is why, under specific conditions, it is more appropriate to test random DNA fragments using analyses sensitive to changes in the sequence, including to spot mutation. These are SSCP and DGGE (denaturation gradient gel electrophoresis). Pulse electrophoresis profiles and RAPD profiles before and after

treatment are one and the same for micrococci, i.e. there are no significant changes in the genome. For *L. Plantarum* the isolates from 49 to 53 and from 68 to 70 had one and the same profiles according to the above analyses. Isolate № 67 was an exception in both analyses /Fig.8/.

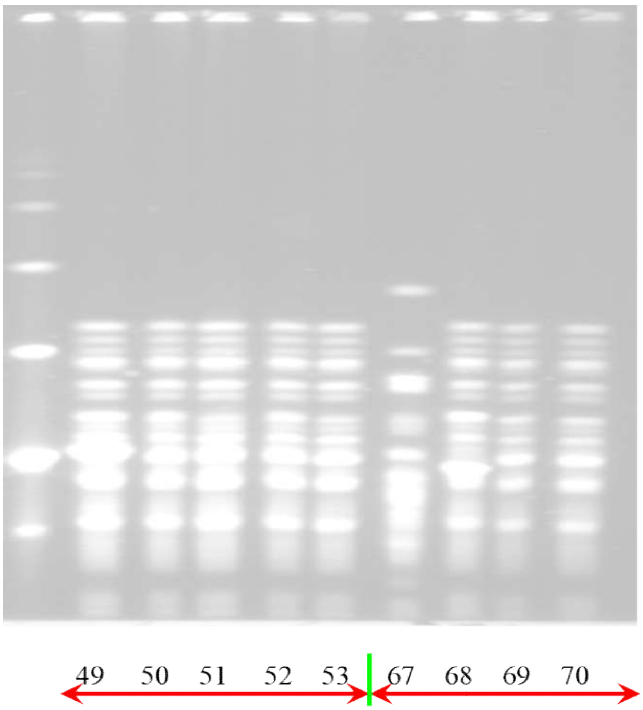

Figure 8. PFGE of *L. plantarum* strains

We believe that the single colony this strain was isolated from, gave a deviation, i.e. after irradiation we observed changes in the genome. Such colonies are cultivated and the bouillon cultures are divided in two. One culture is tested as it is. And all such pairs of test tubes must be compared with each other, i.e are there any changes according to the three analyses? This would not only prove the effect of irradiation affecting DNA of surviving cells, but also the effects killing cells and destroying vital centers. Regarding the third group of isolates, the profiles before and after treatment are one and the same, i.e. there are no significant changes in the genome. Next to the relevant profiles on the pictures are the numbers of the isolates so you can compare them - which isolates are the same. The analyses confirm the biological experimental results, namely that the higher death rate and worse blood indicators of the mice fed on raw-dried sausages with starter 2 are not due to the bacterial factor, but most probably to the fermentation processes in sausages prepared with such start culture. This paper presents the experiments which reveal the fact that irradiation may increase food safety and quality.

Conclusion

- The analyses confirm the biological experimental results, namely that the worse indicators of the mice fed on raw-dried sausages with starter 2 are not due to the bacterial factor, but most probably to the fermentation processes in sausages prepared with such start culture.
- Pulse electrophoresis profiles and RAPD profiles before and after treatment are one and the same for micrococci, i.e. there are no significant changes in the genome.
- For *L. Plantarum* the isolates from 49 to 53 and from 68 to 70 had one and the same profiles.
- Isolate № 67 was an exception in both analyses. We believe that the single colony this strain was isolated from, gave a deviation, i.e. after irradiation we observed changes in the genome.
- The tested meat products do not have any harmful effect a test animals regarding the tested indices
- Ionizing irradiation treatment of meat products had a positive effect and extending their shelf life.

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