ISSN 1062-8738, Bulletin of the Russian Academy of Sciences: Physics, 2018, Vol. 82, No. 6, pp. 740–744. © Allerton Press, Inc., 2018.
Original Russian Text © U.A. Bliznyuk, V.M. Avdyukhina, P.U. Borchegovskaya, V.V. Rozanov, F.R. Studenikin, A.P. Chernyaev, D.S. Yurov, 2018, published in Izvestiya Rossiiskoi Akademii Nauk, Seriya Fizicheskaya, 2018, Vol. 82, No. 6.

Innovative Approaches to Developing Radiation Technologies for Processing Biological Objects

U. A. Bliznyuk[a, *], V. M. Avdyukhina[a], P. U. Borchegovskaya[a], V. V. Rozanov[a], F. R. Studenikin[a], A. P. Chernyaev[a, b], and D. S. Yurov[b]

[a] *Moscow State University, Moscow, 119991 Russia*
[b] *Skobeltsyn Institute of Nuclear Physics, Moscow State University, Moscow, 119991 Russia*
**e-mail: uabliznyuk@gmail.com*

Abstract—Experimental studies on the radiation treatment of food products by various types of ionizing radiation are conducted at Moscow State University's Faculty of Physics. The effect different doses of X-ray radiation have on the biochemical characteristics of potatoes is considered as an alternative to gamma radiation and accelerated electrons. The effect different doses of accelerated electrons have on the microbiological parameters of refrigerated fish products is also considered. Results are presented from studies on the radiation sterilization of bioimplants in combination with chemical action. The proposed technique of combined sterilization based on the effect of an ozone–oxygen mixture and a beam of accelerated electrons allows the radiation dose of bioimplants to be reduced.

DOI: 10.3103/S1062873818060072

INTRODUCTION

Radiation technologies are currently used to solve many scientific and production problems in such areas as health care, the preservation and quality of different agricultural products, the modification of construction and finishing materials, flaw detection, and control of luggage and cargo in container transport. The problems of extending storage life for agricultural products and the sterilization of bioimplants are priority areas in joint studies performed over the past few years by Moscow State University's Faculty of Physics and scientists and technicians from the Research and Training Center of Biomedical Technologies at the Russian National Research Institute of Medicinal and Aromatic Plants (*VILAR*). The innovative physicochemical approaches now under development are proving to be economical and safe for humans, and effective and easy to adapt to practical applications.

AREAS OF APPLICATION

Radiation Treatment of Food Products

One area of research is the development of modern radiation technologies and improving their efficiency in preserving fresh agricultural produce, meat and meat products, and fish products. Studies are now under way on the effect gamma radiation has on different indicators of fresh agricultural produce. Much attention is being given to studying the structural, chemical, and morphological properties of vegetables after irradiation, especially potatoes [1–5]. We know that the nutritional value of potatoes and their organoleptic properties do not change under the effects of gamma radiation in doses of 50 to 150 Gy. These doses can greatly prolong the storage life of potatoes [1–6]. It is important to consider the grade, storage temperature, and processing time during the radiation treatment of vegetable crops [6]. Much more rarely, electron beams in this range of doses are used as an alternative way of halting the germination of potatoes [7–9].

Intensive research is currently being done on the possible use of ionizing radiation to extend the shelf life of fish and meat products. It is known that the shelf life of refrigerated fish ranges from 7 to 9 days, depending on its grade and the conditions of storage. It has been shown that gamma radiation in doses of 1 to 5 kGy extends this time from 12 to 20 days, depending on the conditions of storage [10–17]. The growth of the quantity of pathogenic bacteria and microorganisms is thus slowed at doses as high as 3 kGy. In addition, the size of these populations falls as the dose of radiation is increased, and the storage period is extended to 12–14 days [10, 12, 13]. The development of pathogens is completely blocked at a dose of 5 kGy, and the shelf life is extended to 18–20 days [12, 15]. The use of gamma radiation to extend the storage period of meat and poultry is also being investigated. It is shown that the storage time can be as long as 16 days with irradiation of refrigerated meat products in the range of doses of 10 kGy and higher [18].

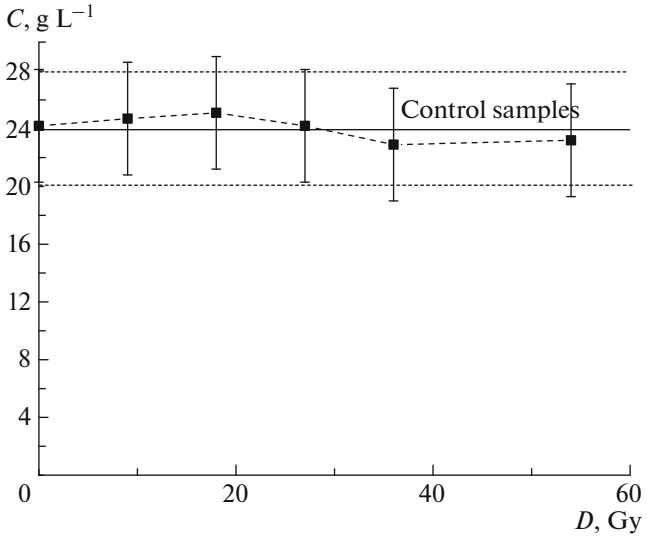

Fig. 1. Dependence of the concentration of protein in potatoes on the dose of radiation.

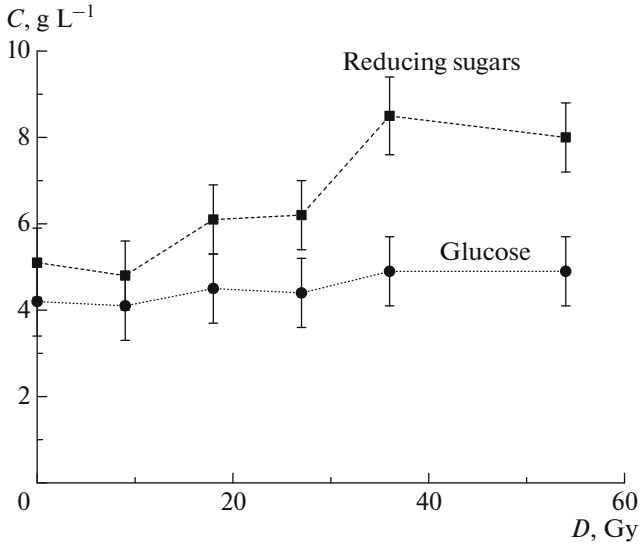

Fig. 2. Dependence of the concentrationsof sugars and glucose in potatoes on the dose of radiation.

The chemical and organoleptic properties of fish and meat products after treatment with gamma radiation are being studied along with their microbiological characteristics. It is known that gamma radiation in doses of up to 3 kGy does not affect the taste or external characteristics of fish products [12, 13, 16, 17]. An increase in the peroxide oxidation of lipids and proteins is observed at doses of gamma radiation of 5 kGy and higher. This alters the relevant organoleptic properties [12, 15]. As with fish products, there is an increase in the peroxidation of lipids and proteins upon the irradiation of beef, pork, and poultry. The taste, color, and smell of products are also changed [18–20].

It is of interest to study the effect X-ray radiation has on the biochemical parameters of fresh agricultural products (potatoes) as an alternative to gamma radiation and accelerated electrons, and to study the effect accelerated electrons have on the microbiological parameters of fish products (refrigerated trout).

Nevskii-grade potato tubers weighing 100–120 g, grown at Russia's Lohr Research Institute of Potato Farming, were selected as objects for studying changes in biochemical parameters of fresh agricultural produce. The potato tubers were irradiated with X-rays from a PUR 5/50 power supply unit and a BSV-23 X-ray tube with a molybdenum anode. The tube's current was 20 mA in all our experiments. The voltage was 50 kV, and the tube's working capacity was 1 kW. The potatoes were irradiated on both sides for 15, 30, 45, 60, and 90 min to ensure uniformity of the radiation's effect. Modeling was done with the GEANT4 program code to estimate the absorbed dose, allowing for the technical characteristics of the employed tube. Calculations showed the exposure times that were used corresponded to doses of 9, 18, 27, 36, and 54 Gy. The experiments were performed at a temperature of 18°C, and the relative humidity was 40–50%.

The protein concentration in potatoes was determined according to Lowry, and the concentrations of reducing sugars and glucose were determined by colorimetric means three days after irradiation. The data was then compared to the corresponding benchmarks. The extract that was centrifuged and used as a working solution was isolated from each potato tuber to measure the above characteristics.

The dependence of protein concentration C in a potato on radiation dose D after treatment with X-rays is shown in Fig. 1. For comparison, the protein concentration in the control (non-irradiated) samples with the corresponding error of measurement is shown by a solid line. We can see that X-rays in the 9 to 54 Gy range of doses produced no appreciable change in the amount of protein in the potato tubers.

Concentration dependences C of reducing sugars and glucose in potatoes on radiation dose D are shown in Fig. 2. The dose of 0 Gy corresponds to the parameters of the control samples. We can see that the amount of reducing sugars did not change and was virtually equal to the control value (5 ± 0.8) g L^{-1} at doses as high as 10 Gy. The concentration of sugars grew at doses of 20 Gy and higher; its maximum value was (8.5 ± 0.9) g L^{-1}. The glucose concentration remained virtually unchnaged in the 9 to 54 Gy range of doses.

Refrigerated trout was chosen as our object for studying the effect accelerated electrons have on the microbiological parameters of fish products. Chunks of refrigerated trout 0.7 cm thick were irradiated with a beam of accelerated electrons obtained from the UELP-1-25-T-001 continuous electron beam accelerator with an energy of 1 MeV and a mean beam power

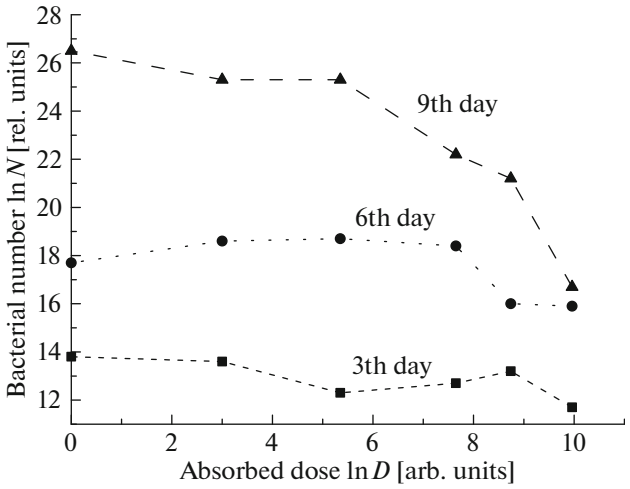

Fig. 3. Dependences of the QMAFAnM bacterial number (ln*N*) in samples on the dose of radiation (ln*D*), measured on the 3rd, 6th, and 9th day after irradiation.

of 25 kW. Samples were irradiated at a temperature of 18°C, with five different doses from both sides to ensure uniformity of treatment. The quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) in the radiated and non-irradiated samples was then measured in CFU/g on the 3rd, 6th, and 9th days after irradiation. The storage temperature during this period was 6°C.

Computer simulations using the GEANT4 code were performed to estimate the absorbed dose in trout, allowing for the technical characteristics of the UELP-1-25-T-001 accelerator and the parameters of irradiation. Calculations showed the trout was irradiated with doses of 20 Gy, 200 Gy, 2 kGy, 6 kGy, and 20 kGy.

Figure 3 shows the dependences for the quantity of mesophilic aerobic and facultative anaerobic microorganisms (ln *N*) measured on the 3rd, 6th, and 9th days after irradiation on the absorbed dose (ln *D*), calculated using the program code.

Our experiment showed that the quantity of microorganisms fell as the dose of radiation grew in the range of 20 Gy to 20 kGy under the effect of an accelerated electron beam with an energy of 1 MeV. In addition, the longer the time after irradiation, the greater the observed difference between the non-irradiated and radiated samples.

Radiation Sterilization of Bioimplants

Radiation is used effectively to sterilize bioimplants, especially plastic bone material. This technological procedure is increasingly in demand due to the steady rise in recent years in the need for the bone materials used in reconstructive surgery [21, 22]. Sterilization holds an important place in the technological chain of manufacturing bone implants, since it does not matter how perfect the transplant material is if its introduction into the recipient organism is not completely safe [23, 24].

Historically, the technologies of such sterilization developed from the traditional means of hyperthermia (moist heat sterilization). However, such treatment is not effective for biomaterials because there is denaturation of protein upon prolonged high-temperature exposure. At the same time, it should be noted that a number of authors have proposed original means for the thermal inactivation of viruses in bone implants [25–27]. Chemical treatment of biomaterials (in a gas medium or in special solutions [28, 29]) was long considered to be better. At the same time, some researchers have claimed [28] that treating bone samples with different liquid reagents at ideal concentrations under optimum regimes does not degrade the useful properties of implants, while other researchers directly note the low effectiveness of chemical sterilization in the fight against spores and viruses [30].

The use of a gas reagent (ethylene oxide) is accompanied by even more contradictory opinions of specialists, from unconditional support [29, 31, 32] to categorical rejection because it does not meet safety requirements [33] or ensure preservation of the osteinductive properties of transplants [34, 35].

In recent years, radiation sterilization of bioimplants has been considered most effective. Two main methods are used today: sterilization by ^{60}Co gamma radiation [36, 37] and by fast electron beams on accelerators with energies of 5–10 MeV [38]. The use of ionizing radiation offers a number of advantages, due to its high penetrating power and weak heating of treated objects. In addition, it can be used to sterilize objects placed in hermetically sealed packaging, reducing the risk of secondary contamination.

Both means have been used on an industrial scale for more than 50 years [39] and are in constant development and competition. The focus in the late 1950s was originally on developing ways of sterilizing medical items using beams of fast electrons. Industrial gamma technologies predominated in the next decade. Interest in electron-beam devices has been revived in recent years. In certain applications, electron-beam devices offer some advantages over gamma-ray sterilization:

—There are no problems with the use, transportation, or storage of radioactive materials.

—Production process can be combined for continuous processing.

—The much shorter time of irradiation ensures higher productivity.

Both effects are in this case characterized by an almost equivalent biological effect [40] that is determined by the amount of an absorbed dose. The same parameter is crucial for the effectiveness of the sterilization process. A value of 25 kGy is usually taken as

the standard dose [41]. However, there is evidence that radiation exposure with dose of up to 60 kGy is necessary for the effective inactivation of HIV [42]. At the same time, many researchers cite data that irradiation with absorption doses of 15 kGy or higher lead to substantial changes in the mechanical characteristics of bioimplants and their morphology [43, 44]. The impact of high-energy radiation is also detrimental to morphogenetic proteins, and eventually reduces the osteinductive properties of implants severely. A working range of radiation sterilization from 15–25 to 25–35 kGy has established as a compromise solution in an number of countries [21, 41, 45], but the task of reducing the dose remains on the agenda.

One way of solving this problem is to use combined technologies based on the synergy of the two-step combined sterilizing effect of different physicochemical factors [46–48]. Existing technological approaches call for a sterilized sample to be exposed to chemical action in the liquid or gaseous phase in the first stage, and then to radiation in the second stage. The separate impact of each factor is not enough for complete sterilization of the object, but together they reinforce each other. This not only ensures sterilization but also allows us to reduce the dose of radiation considerably. Our proposed technology for the combined sterilization of bone implants is based on the combined action of a ozone–oxygen mixture and radiation treatment using a beam of fast electrons. This technique has been shown to achieve the required sterility of samples at doses of 11–13 kGy. Such an absorbed dose allows less expensive options (e.g., X-rays) to be used as sources of ionizing radiation [49]. This technology is protected by a patent of the Russian Federation [50].

CONCLUSIONS

Laboratory research and clinical experience show that the development of innovative approaches to the radiation treatment of biological objects, especially products of the agroindustrial complex and bioimplants, is not only relevant but also leads to promising new lines for the application and improvement of radiation technologies.

REFERENCES

1. Nouri, J. and Toofanian, F., *Pak. J. Biol. Sci.*, 2001, vol. 4, p. 1275.
2. Burton, W.G. and Hannan, R.S., *J. Sci. Food Agric.*, 1957, vol. 8, p. 707.
3. Ghanekar, A.S., Padwal-Dessi, S.R., et al., *J. Agric. Food Chem.*, 1983, vol. 31, p. 1009.
4. Frazier, M.J., et al., *Am. J. Potato Res.*, 2006, vol. 83, p. 31.
5. Rezaee, M., et al., *J. Agric. Sci. Technol.*, 2011, vol. 13, p. 829.
6. Neelma, M., et al., *Pak. J. Life Soc. Sci.*, 2015, vol. 13, no. 3, p. 153.
7. Hayashi, T. and Todoriki, S., in *Proc. FNCA Workshop on Application of Electron Accelerator*, Takasaki, 2002, p. 100.
8. Hayashi, T. and Todoriki, S., *Radiat. Phys. Chem.*, 2000, vol. 57, p. 253.
9. Alimov, A.S., Bliznyuk, U.A., Borchegovskaya, P.U., Varzar, S.M., Elansky, S.N., Ishkhanov, B.S., Litvinov, U.U., Matveychuk, I.V., Nikolaeva, A.A., Rozanov, V.V., Studenikin, F.R., Chernyaev, A.P., Shvedunov, V.I., and Yurov, D.S., *Bull. Russ. Acad. Sci.: Phys.*, 2017, vol. 81, no. 6, p. 743.
10. Jeevanandam, K., Kakatkar, A., et al., *Food Res. Int.*, 2001, vol. 34, p. 739.
11. Dymsza, H.A., Lee, C.M., et al., *J. Food Sci.*, 1990, vol. 55, p. 1745.
12. Moini, S., Tahergorabi, R., et al., *J. Food Prot.*, 2009, vol. 72, p. 1419.
13. Chouliara, I., Savvaidis, I.N., et al., *J. Sci. Food Agric.*, 2005, vol. 85, p. 779.
14. Arvanitoyannis, I.S., Stratakos, A., et al., *Crit. Rev. Food Sci. Nutr.*, 2008–2009, vol. 49, p. 68.
15. Hocaoğlu, A., Sükrü Demirci, A., et al., *Radiat. Phys. Chem.*, 2012, vol. 81, p. 1923.
16. Ahmed, I.O., Alur, M.D., et al., *Int. J. Food Sci. Technol.*, 1997, vol. 32, no. 4, p. 325.
17. Lakshmanan, R., Venugopal, V., et al., *Food Res. Int.*, 1999, vol. 32, p. 707.
18. Lefebvre, N., Thibault, C., et al., *Meat Sci.*, 1994, vol. 36, p. 371.
19. Heath, J.L., Owens, S.L., et al., *Poultry Sci.*, 1990, vol. 69, p. 313.
20. Ahn, D.U., Olson, D.G., et al., *J. Food Sci.*, 1998, vol. 63, no. 1, p. 15.
21. Dziedzic-Goclawska, A., Kaminski, A., et al., *Cell Tissue Banking*, 2005, vol. 6, p. 201.
22. Rozanov, V.V., Matveichuk, I.V., et al., *Tekhnol. Zhivykh Sist.*, 2015, vol. 12, no. 4, p. 59.
23. Lekishvili, M.V., Techniques for fabrication of bone plastic material for reconstructive surgery (experimental study), *Doctoral (Med.) Dissertation*, Moscow, 2005.
24. Panteleev, V.I. Rozanov, V.V., et al., *Biomed. Radioelektron.*, 2013, no. 2, p. 3.
25. Le Huec, J.C., *Chirurgie*, 1992, vol. 118, nos. 6–7, p. 397.
26. Kuhne, J.H., Refior, H.J., et al., *Z. Orthop. Ihre Grenzgeb.*, 1994, vol. 132, no. 2, p. 102.
27. Dormont, D., *Transplant. Proc.*, 1996, vol. 28, no. 12, p. 2931.
28. Savel'ev, V.I., in *Poluchenie i klinicheskoe primenenie demineralizovannykh kostnykh transplantatov* (Fabrication and Clinical Use of Demineralized Bone Implants), Leningrad: Nauchno-Issled. Inst. Travmatol. Ortop., 1987, p. 4.
29. Kakiuchi, M., Ono, K., et al., *Int. Orthop.*, 1996, vol. 20, no. 3, p. 142.
30. Pugliese, G. and Favero, M.S., *Infect. Control Hosp. Epidemiol.*, 2000, vol. 21, no. 8, p. 549.
31. Jackson, D.W., Windler, G., et al., *Am. J. Sports Med.*, 1990, vol. 18, no. 1, p. 1.

32. Tshamala, M., Cox, E., et al., *Vet. Immunol. Immunopathol.*, 1999, vol. 69, no. 1, p. 47.
33. Danielson, N.E., *Sterilization of Medical Products*, Johnson & Johnson, 1991, p. 194.
34. Thorén, K. and Aspenberg, P., *Clin. Orthop. Relat. Res.*, 1995, vol. 318, p. 259.
35. Russell, J.L. and Block, J.E., *Orthopedics*, 1999, vol. 22, no. 5, p. 524.
36. *Trends in Radiation Sterilization of Health Care Products*, Vienna: International Atomic Energy Agency, 2008.
37. Singh, R., Singh, D., et al., *World J. Radiol.*, 2016, vol. 8, p. 355.
38. Beck, J.A., *Radiat. Phys. Chem.*, 2012, vol. 81, p. 1236.
39. Baba, T., Kaneko, H., et al., *Radiat. Phys. Chem.*, 2004, vol. 71, p. 207.
40. Tallentire, A., Miller, A., et al., *Radiat. Phys. Chem.*, 2010, vol. 79, p. 701.
41. Perova, N.V., Dovzhik, I.A., et al., Abstracts of Papers, *V Vserossiiskii simpozium "Aktual'nye voprosy tkanevoi i kletochnoi transplantologii"* (V All-Russian Symp. "Topical Problems of Tissue and Cell Transplantology"), Ufa, 2012, p. 99.
42. Campbell, D.G. and Li, P., *ANZ J. Surg.*, 1999, vol. 69, p. 517.
43. Zhang, Y., Homsi, D., et al., *Spine*, 1994, vol. 19, p. 304.
44. Shangina, O.R. and Nigmatullin, R.T., *Morfologiya*, 2006, vol. 129, no. 3, p. 44.
45. *Standards for Tissue Banking*, Hornicek, F.J., Woll, J.E., and Kasprisin, D., Eds., McLean: American Association of Tissue Banks, 2002, 10th ed.
46. Matveichuk, I.V., Rozanov, V.V., et al., *Vopr. Biol., Med. Farm. Khim.*, 2013, vol. 11, no. 11, p. 92.
47. Rozanov, V.V., Bykov, V.A., et al., *Med. Al'm.*, 2013, no. 3, p. 24.
48. Savel'ev, V.I., Bulatov, A.A., and Rykov, Yu.A., RF Patent 2356224, *Byull. Izobret.*, 2009, no. 15.
49. Tallentire, A. and Miller, A., *Radiat. Phys. Chem.*, 2015, vol. 107, p. 128.
50. Matveichuk, I.V., Rozanov, V.V., Gordonova, I.K., et al., RF Patent 2630464, *Byull. Izobret.*, 2017, no. 25.

Translated by I. P. Obrezanova