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IMPACT OF 1 MeV ACCELERATED ELECTRONS ON GROWTH AND SURVIVAL RATE OF *ESCHERICHIA COLI* BACTERIA AND *ASPERGILLUS FUMIGATUS* FUNGUS

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Abstract. *A study was carried out on the effect of 1 MeV accelerated electrons on the survival rate of suspensions of Escherichia coli bacteria and suspensions of Aspergillus fumigatus fungi at various initial concentrations and followed by plating on various nutrient media after irradiation. The samples were irradiated in the dose range from 0.15 kGy to 4 kGy. It was established that the concentrations of viable bacterial and fungal cells decreased nonlinearly with radiation dose. The doses required to reduce populations by a factor of 10 ranged from 0.20 kGy to 0.56 kGy for Escherichia coli at initial concentrations of 10^3 CFU/g to 10^5 CFU/g when plated on agar Thioglycollate medium; 1.28 kGy and 1.23 kGy for Aspergillus fumigatus at an initial concentration of 10^6 CFU/g when plated on Sabouraud medium and Modified Czapek-Dox medium, respectively.*

Keywords: *radiation processing, electron radiation, radiation dose, D_{10} dose, Escherichia coli bacteria, Aspergillus fumigatus fungi, survival rate, radioresistance*

1. INTRODUCTION

A wide range of microorganisms, including pathogenic and opportunistic bacteria, mold fungus, harmful viruses, and parasites reproduce in food, which is a favorable environment for their growth. The World Health Organization estimates that 420 000 people die each year from foodborne illnesses, making it urgently necessary to ensure food safety throughout every stage of food production and transportation worldwide [1].

Radiation processing techniques are getting highly demanded in the food and agricultural industries across the globe as a means of preserving food quality and ensuring its microbiological safety. According to ISO 14470:2011 [2], the use of accelerated electron beams with energies up to 10 MeV, bremsstrahlung and X-rays with energies up to 5 MeV, and gamma radiation from ^{60}Co and ^{137}Cs radioisotopes makes it possible, with properly selected irradiation parameters, to ensure the microbiological safety of food products with preservation of their nutritional and taste qualities [3–5].

Around the world, research is being done to study the effect of ionizing radiation on the microbiological, biochemical, and organoleptic characteristics of food products in order to determine radiosensitivity of bacteria [6–11].

The viability of the microorganisms that are most frequently found in meat and fish products is under investigation. They include mold fungus of *Aspergillus, Penicillium, Cladosporium, Cryptococcus* genera, etc., and opportunistic and pathogenic bacteria of *Escherichia coli, Salmonella, Listeria* genera, etc. [12].

The aim of the study is to investigate the effect of 1 MeV accelerated electrons beam on the survival rate of *Escherichia coli* bacteria and *Aspergillus fumigatus* fungi at various initial concentrations, and on their growth after plating in various nutrient media.

2. MATERIALS AND METHODS

2.1. Stages of research

Escherichia coli bacteria suspensions in saline at concentrations of 10^3, 10^4, and 10^5 CFU/g, and suspensions of *Aspergillus fumigatus* spores in saline at a concentration of 10^6 CFU/g were exposed to accelerated electron radiation at doses ranging from 0.15 kGy to 4 kGy. The bacteria were plated on the agar Thioglycollate medium's surface two hours after the irradiation; the fungal suspension samples were plated on the Sabouraud nutrient medium and the Modified medium, which had the salt background of the Czapek-Dox medium but with 2% collagen in place of sucrose. On the second day after incubation, the number of viable cells in samples of bacteria and fungi irradiated at various doses was counted.

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2.2. Object of study

The objects of study were opportunistic *Escherichia coli* bacteria (reference strain of the American Type Culture Collection (ATCC) – *Escherichia coli* ATCC 25922), grown on agar Thioglycollate medium for a day at 37 °C under sterile conditions, and spores of *Aspergillus fumigatus* fungi, grown for three days at (25–27) °C on Sabouraud agar medium. Samples of bacteria and fungi were provided from the collection of pure cultures of the All-Russian Scientific Research Institute of Medicinal and Aromatic Plants.

The selection of *Escherichia coli* bacteria for irradiation was carried out as follows: using a liquid Thioglycollate medium, *E. coli* bacteria were removed from the surface of the agar medium; the suspensions were then diluted to achieve bacterial concentrations of 10^3, 10^4, and 10^5 CFU/g in accordance with the McFarland standard turbidity [13]. The concentration of viable bacteria in the suspension was determined by successive 10-fold dilutions and inoculation on agar Thioglycollate medium. The initial concentrations of bacteria in suspensions were (4.2 ± 0.4) 10^3 CFU/g, (4.3 ± 0.6) 10^4 CFU/g, and (5.0 ± 0.7) 10^5 CFU/g.

The micromycete *Aspergillus fumigatus* spores were removed from the agar medium using Sabouraud's liquid medium; the concentration of the spores in the suspension was then evaluated using a series of 10-fold dilutions and inoculation on an identical-type agar medium. Initial *A. fumigatus* spore concentrations in suspensions were (3.7 ± 0.3) 10^6 CFU/g for the Sabouraud medium and (2.6 ± 0.2) 10^6 CFU/g for the Modified medium, which had the salt background of the Czapek-Dox medium with the addition of 2% collagen in place of sucrose.

Suspensions of bacteria and fungi, 0.5 ml each, were added in sterile 2-ml Eppendorf test tubes. Experiments were conducted in two iterations using a total of 36 and 28 pieces of prepared samples of bacterial and fungal suspensions, respectively.

2.3. Irradiation of samples

Samples of bacteria and fungi were irradiated using a UELR-1-25-T-001 electron accelerator (SINP MSU, Russia) with maximum energy of 1 MeV and beam power of 25 kW. Six pieces of suspension samples were set out on a duralumin plate that was 12 cm away from the beam exit. During each irradiation session, the irradiation time, beam current, and charge absorbed by the plate were recorded. The irradiation scheme is described in [14]. The average beam current was (200 ± 5) nA. Irradiation was carried out at a temperature of 20 °C.

The dosimeter Fricke solution was used to calculate the dose that the samples absorbed. The dosimeter solution irradiation scheme corresponded to the experimental samples irradiation scheme. By adjusting the charge absorbed by the duralumin plate and the exposure time, the dose absorbed by the samples was varied. For the *E. coli* bacteria radiation doses were 0.15 kGy, 0.3 kGy, 0.6 kGy, 1 kGy, and 4 kGy; for the *A. fumigatus* fungus radiation doses were 0.25 kGy, 0.5 kGy, 1 kGy, 1.5 kGy, 2 kGy, and 3 kGy. The dose rate was (3.7 ± 0.5) Gy/s.

The uniformity of the dose distribution over the volume of the suspension was evaluated by computer simulation using the Geant4 toolkit [15]. The ratio between the minimum and maximum absorbed doses in the layer, or the uniformity of irradiation, was 75 %. When irradiated with accelerated electrons, the non-uniformity of the dose distribution over the sample volume is inevitable, that must be taken into account when choosing the optimal parameters for processing foodstuffs with electron beam.

2.4. Microbiological analysis

After being irradiated, the suspensions were successively diluted in the ratios of 1:2, 1:10, 1:100, 1:1000, and 1:10000 for the irradiated and non-irradiated control samples. For each dilution, 0.1 ml of the suspension was then taken and applied to a nutrient medium (agar Thioglycollate medium for bacteria, Modified medium, and Sabouraud medium for fungi). After two days of incubation of bacteria at 37 °C and micromycetes at (25-27) °C, the number of viable cells in CFU/g in the suspension volume was determined. The choice of two nutrient media for the cultivation of *A. fumigatus* is associated with the previously obtained data on the proteolytic activity of the fungus, namely, the ability to grow and form lysis zones during surface cultivation on media with a complete replacement of easily metabolized carbohydrate (sucrose) in Czapek-Dox medium by insoluble proteins (collagen) [16].

A sample dilution procedure for estimating the number of microorganisms when plated on various nutrient media is shown in Figure 1. The standard method was used to count the number of microorganisms [17].

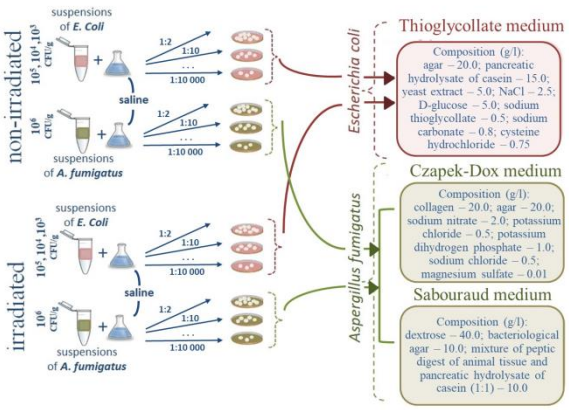

Figure 1. Scheme of cultivation and isolation of *Escherichia coli* bacteria and *Aspergillus fumigatus* fungi

2.5. Evaluation of the survival rate of bacteria and fungi after irradiation

The needed value of the dose D_{10} to reduce the number of viable cells by 10 times was computed using the formula:

$$D_{10} = \frac{D}{Log_{10}N - Log_{10}N_0} \qquad (1)$$

where D is the radiation dose, N_0 is the initial concentration of microorganisms, N is the concentration of microorganisms after irradiation at dose D. The direct dependence of $Log_{10}(N/N_0)$ on the dose D is used to derive the D_{10} value [6]. This value was used to evaluate the survival rate of bacteria and fungi

after being exposed to various doses of accelerated electron radiation.

3. Results and discussion

3.1. Dose-effect curves for Escherichia coli bacteria

The data on the results of a microbiological study of the effect of accelerated electrons at different doses on the number of viable cells of *Escherichia coli* bacteria are presented in Table 1.

Table 1. Concentration of viable *Escherichia coli* bacteria cells at different initial concentrations and irradiation doses

Dose	*N*, CFU/g		
Control	(**4.2** ± 0.4)·**10³**	(**4.3** ± 0.6)·**10⁴**	(**5.0** ± 0.7)·**10⁵**
0.15 kGy	(**0.28** ± 0.03)·**10³**	(**0.3** ± 0.5)·**10⁴**	(**0.18** ± 0.03)·**10⁵**
0.3 kGy	(**0.18** ± 0.03)·**10³**	(**0.08** ± 0.06)·**10⁴**	(**0.22** ± 0.03)·**10⁵**
0.6 kGy	(**0.042** ± 0.005)·**10³**	(**0.037** ± 0.007)·**10⁴**	(**0.09** ± 0.01)·**10⁵**
1 kGy	ND[2]	ND	(**0.020** ± 0.005)·**10⁵**
4 kGy	ND	ND	ND

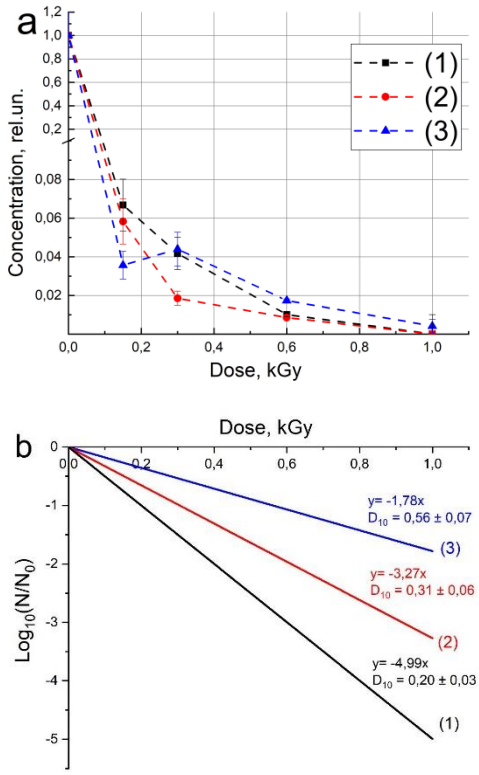

Figure 2. Dependences of the relative concentration of *Escherichia coli* (a); and $Log_{10}(N/N_0)$ (b) on the radiation dose for initial concentrations of bacteria 10^3 CFU/g (1), 10^4 CFU/g (2), and 10^5 CFU/g (3).

A non-linear decrease in the concentration of viable bacterial cells in suspensions occurs with an increase in the irradiation dose (Tab. 1). The dependences of viable cell concentrations in bacterial suspensions normalized to the initial bacterial concentrations on the irradiation dose (Fig. 2a). On the one hand, in the suspension with an initial concentration of 10^5 CFU/g, viable cells were detected at (0.020 ± 0.005)·10^5 CFU/g, on the other hand, no viable cells were detected in the suspensions with initial concentrations of 10^3 and 10^4 CFU/g when irradiated at a dose of 1 kGy. Irradiation at 4 kGy completely suppressed *E. coli* bacteria for all initial concentrations studied.

The dose dependences of $Log_{10}(N/N_0)$ from which D_{10} values were calculated for different initial concentrations of bacteria are shown in Figure 2b. The D_{10} value rises from (0.20 ± 0.03) kGy to (0.56 ± 0.07) kGy with an increase in the initial concentration of bacteria from 10^3 CFU/g to 10^5 CFU/g. At an initial concentration of 10^4 CFU/g, the D_{10} value was (0.31 ± 0.06) kGy. As a result, the required dose to reduce the population of bacteria by 10 times increases by 1.5 to 2 times for every 10-fold increase in the initial concentration of microorganisms.

3.2. Dose-effect curves for Aspergillus fumigatus fungi

Data on the results of a microbiological study of the effect of accelerated electrons at different doses on the concentration of *Aspergillus fumigatus* fungi are presented in Table 2.

Table 2. Concentration of viable cells of *Aspergillus fumigatus* fungi on the dose of electron irradiation with plating on Sabouraud and Modified media.

Dose	Sabouraud medium	Modified medium
	N, CFU/g	
Control	(**3.7** ± 0.3)·**10⁶**	(**2.6** ± 0.2)·**10⁶**
0.25 kGy	(**3.1** ± 0.3)·**10⁶**	(**2.1** ± 0.1)·**10⁶**
0.5 kGy	(**1.1** ± 0.2)·**10⁶**	(**0.81** ± 0.03)·**10⁶**
1 kGy	(**0.58** ± 0.06)·**10⁶**	(**0.42** ± 0.03)·**10⁶**
1.5 kGy	(**0.22** ± 0.03)·**10⁶**	(**0.16** ± 0.01)·**10⁶**
2 kGy	(**0.12** ± 0.02)·**10⁶**	(**0.06** ± 0.01)·**10⁶**
3 kGy	(**0.10** ± 0.01)·**10⁶**	(**0.05** ± 0.01)·**10⁶**

Decreasing in the concentration of viable fungal cells was seen in relation to radiation at doses ranging from 0.25 kGy to 3 kGy, both during growth on carbohydrate-rich Sabouraud medium and on Modified Czapek-Dox medium (Fig. 3a). Irradiation at a maximum dose of 3 kGy did not completely inhibit the growth of the fungus. The results demonstrate that after being irradiated at various doses, there were no statistically differences in the concentrations of viable cells grown on various nutritional media.

[2] ND – Not Detected

Under irradiation at doses from 0.25 kGy to 1.5 kGy, the decrease in the concentration of *A. fumigatus* during cultivation on a substrate that is difficult to utilize is comparable with the control values and ranges from 26 % to 32 %; a further increase in the dose causes an increase in this indicator by 1.5 times. Apparently, the obtained results indicate that at high doses of radiation, the synthesis of collagenolytic proteases by the fungus is disrupted, due to the activity of which the micromycete grows on a Modified medium.

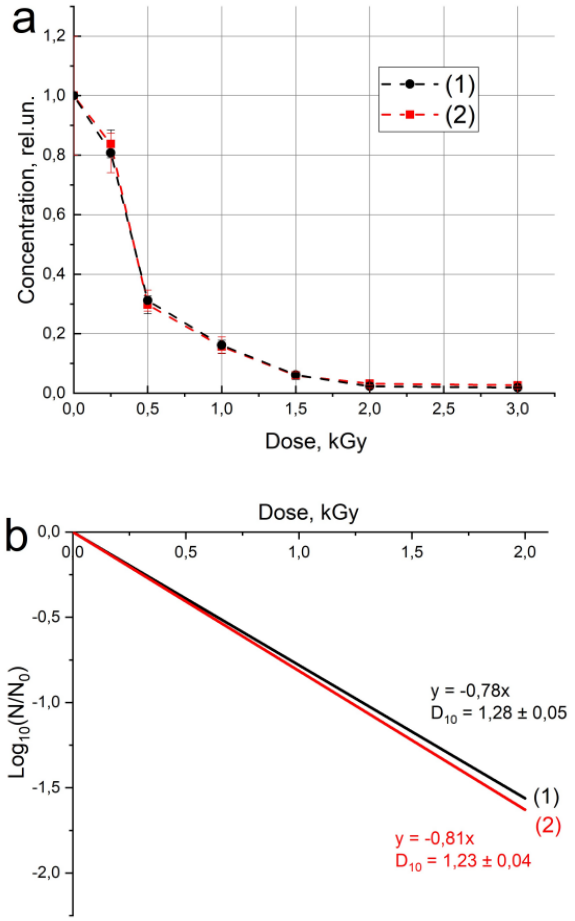

Figure 3. Dependences of the relative concentration of *Aspergillus fumigatus* (a) and $Log_{10}(N/N_0)$ (b) on the radiation dose after plating on various nutrient media: Sabouraud medium (1) and Modified medium (2).

The D_{10} dosage values coincided within the measurement error for both nutrient media and amounted to (1.28 ± 0.05) kGy and (1.23 ± 0.04) kGy for the Sabouraud medium and Modified medium, respectively (Fig. 3b).

3.3. Comparison study

In the works [6,7,8,18], studies are carried out on the effects of gamma radiation produced by radioactive isotopes ^{137}Cs and ^{60}Co and accelerated electrons on chilled and frozen meat products: pig, poultry, chicken, and ground beef containing *E. coli* bacteria. The D_{10} values increase from 0.22 kGy to 0.63 kGy at concentrations between 10^5 CFU/g and 10^9 CFU/g, depending on the product's kind, storage temperature, and initial bacterial concentration. In our study, the D_{10} dose increased from 0.20 kGy to 0.56 kGy with an increase in the initial concentration of bacteria from 10^3 CFU/g to 10^5 CFU/g, which is in good agreement with the data of other authors.

Escherichia coli bacteria were irradiated by 10 MeV accelerated electrons in [9], the D_{10} value was 0.27 kGy, which is almost 2 times lower than the results from this study. This difference can be attributed to different electron energies, distributions of linear energy losses, and spatial distributions of ionization events in the volume of irradiated samples.

The D_{10} values obtained in this study at an initial fungal concentration of 10^6 CFU/g are lower than the D_{10} values discovered [10], when irradiating by ^{60}Co radioisotope two strains of the *Aspergillus alutaceus* and *Aspergillus flavus* fungi with an initial concentration of 10^5 CFU/g in saline. Accordingly, the needed dose to reduce the fungal population by a factor of 10 or more depends on the initial concentration of fungi, which is consistent with the results of irradiation of bacteria obtained in this study. It is also discussed in the literature that the fungal strain [19], the type of nutrient medium [11,20], and the type of source [21] can influence the inactivation of fungi.

4. Conclusion

Based on the obtained results the efficiency of food radiation processing can be influenced by both the initial contamination of microorganisms and their strains, as well as the type of product, storage temperature, source of ionizing radiation, etc.

Experimental evidence shows that a higher initial contamination of the product requires a higher dosage of radiation to suppress the concentration of microorganisms by N times. However, at high doses, a change in the product's chemical composition can be observed, thus, to extend the shelf life of products, treatment with ionizing radiation is more effective for fresh products with a lower initial contamination.

It has been shown that *Aspergillus fumigatus* fungi are more radioresistant to accelerated electrons than *Escherichia coli* bacteria. It must be taken into account when choosing the radiation dose when performing food radiation processing with different microbiological composition.

During irradiation with accelerated electrons, the non-uniformity of the effect of radiation on biological objects, in particular bacteria and fungi, is inevitable, which must be taken into account when choosing the optimal parameters for processing foodstuffs with electron beam.

It is interesting to study the survival rate of microorganisms under irradiation in various nutrient media, as well as to monitor their concentration over time after radiation processing at various doses.

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