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Surface Disinfection Of Chicken Eggs By Nanosecond Electron Beam.

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ABSTRACT

Sterilization of food products by ionizing radiation has a great potential. The technology of food irradiation can be improved to reduce the negative impact on the quality and cost of sterilization. Irradiation of biological objects was carried out using a pulsed repetitive nanosecond accelerator URT-0.5 (electron energy up to 500 keV, pulse width 50 ns, pulse repetition frequency up to 200 ps). The distribution of absorbed dose (AD) in the depth of polyethylene was determined by a gray wedge. Measurement of the AD generated by nanosecond electron beam (NEB) was made using a film dosimeter and thermoluminescent dosimeters, TLD-500. The results lead to the conclusion that irradiation of the electron beam with an AD 5 kGy level is sufficient for complete disinfection of the egg surface. AD inside food product will not exceed 8 cGy due to bremsstrahlung.

Keywords: Disinfection, nanosecond electrons beam, bremsstrahlung, dosimetry, sterilization, Salmonella, hens' egg

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INTRODUCTION

At present, the main way to reduce the microbial contamination of foods is by heat treatment. Therefore, heat pasteurization is widely used to increase the shelf life of foods, followed by cooling to temperatures at which the multiplication of microorganisms is difficult. However, thermal sterilization leads to irreversible changes in the properties of raw materials, which is not always acceptable. Applied chemical methods of processing food lead to the same result, and use many preservatives.

It is known that one of the indicators of the quality of eggs is the purity of the shell. However, the presence of contamination by microorganisms not only makes the appearance of the eggs unattractive, but also facilitates the penetration of microorganisms through pores in the shell egg into its content, which leads to rapid deterioration of the eggs, and also puts them at risk of Salmonella infection.

Washing improves the appearance of the eggs, but sharply reduces their storage stability, so it is usually used in the food industry before the eggs are broken up [1]. Such washing results in the opening of pores in the shell, through which the microorganisms can penetrate the egg. It also requires a hot water flow (~ 80°C) and chemically disinfecting substances (3.2 % hydrogen peroxide), which greatly increases environmental pollution by toxic waste [2].

An alternative is radiation sterilization, due to the universality of the harmful effects of ionizing radiation on any biological objects. In this way, the absorbed dose (AD) of radiation sterilization (regardless of the type of radiation) does not exceed 25 kGy [3].

The irradiation of the foods could be accompanied by a variety of chemical reactions that may transform the organoleptic properties of the products and decreace quility of food. Thus, it is necessary to set limits of the AD for the irradiation of various products.

For example, for fresh eggs, a level of AD 3 kGy is recommended, which is close to the AD level for inactivation of the bacteria of the Salmonella group [4]. Irradiated foods are marked with a special "radura" sign, so that the buyer can choose whether to use irradiated products or nor. Unfortunately, radiation phobia is of great importance in consumer choice.

In our view problems of the microbiological contamination of food and consumer sentiment could be solve with the following promising approaches.

Firstly, by proper electron energy selection, to choose such an AD distribution profile within the product that will destroy, upon irradiation, all kinds of microbes, including pathogenic ones on surface and doesnt significant influence on the inside of the food product. For example, AD will be maximum at both on the egg's shell surface and in its pores, as well as in the air chamber up to the under-shell membranes. In that way, there will be practically no exposure of the protein itself.

Secondly, irradiation will produced ozone, which will also contribute to the disinfection of the surface, especially by irradiation of eggs sealed in plastic containers. It will be useful to sterilize the eggs after packaging by the radiation itself, as well as by the creation of ozone at a concentration level lethal to microorganisms in the packaging – radiation-chemical sterilization [5]. At the same time, it is possible to adjust the AD distribution profile within the egg in such a way that its protein is not irradiated by electrons at all. Presence of sealed plastic containers allows us to solve the problem of re-insemination of eggs during storage after irradiation.

Both ways have their merits. Moreover, in the actual technological process, the two ways can be combined in different proportions.

One of the main the disadvantage of irradiation sterilization is its high cost and greater risk for the working staff. However we could significantly reduced risks by optimizing the radiation source.

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At present, nanosecond electronic accelerators for technologies have been developed and manufactured [6], which significantly reduce the cost of the radiation source, as well as the costs of radiation protection of personnel.

In addition, nanosecond electron beam (NEB) has a high biological effect [7]. This will allow the AD magnitude of the NEB to be reduced by the order of 2 or 3 times, which will increase the efficiency of the method while leaving the energy consumption and material costs the same.

A feature of the NEB spectrum is the availability of a much larger volume of energy with low energy. allows us to obtain the desired AD distribution profile within the product (Figure 1), that is a positive properties.

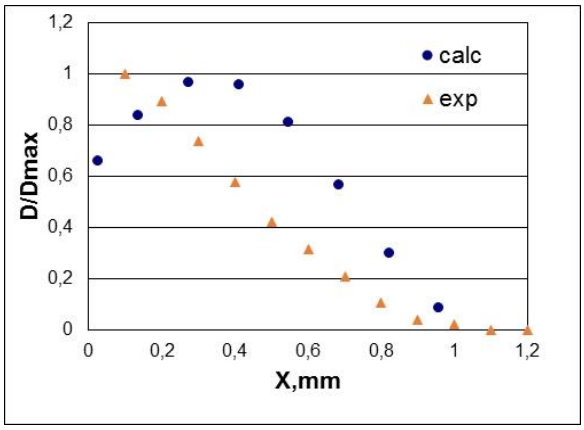

Fig 1: Measured experimentally and calculated distribution of absorbed dose (AD) in the depth in the polyethylene.

MATERIALS AND METHODS

Ionization radiation sources and dosimetry control

The exposure experiments on eggs were carried out by a pulsed repetitive nanosecond accelerator URT-0.5 [8] (electron energy up to 500 keV, pulse width about 50 ns, pulse repetition rate up to 200 pps).

AD was tested by a film dosimeter CO AD (F) R-5/50 [9] covered with polythene layers of varying thickness (up to 600 microns). AD measurements on the film dosimeters were conducted by determining the density of darkening of a spectrophotometer PE 5400VI, followed by recalculation of the calibration lines.

Unfortunately, completely avoid the irradiation of egg interior part is impossible, since bremsstrahlung is induced by absorption of the electrodes, which makes a major contribution to the AD created inside the egg.

Thus, the main aim of this study was to investigate the profile of AD distribution on the surface and inside the egg from the electron beam and bremsstrahlung.

To determine the distribution of AD bremsstrahlung inside hens' eggs, thermoluminescent dosimeters (TLD) TLD-500 (diameter of 5 mm and a thickness of 1 mm) based on aluminum oxide doped with carbon were used.

AD measurement was carried out by a hardware system to highlight TLD dosimeters. Thermoluminescence lines were recorded by a special automatic apparatus at a heating rate of 2 K/s [10]. The signal was detected by a photomultiplier FEU-142 with reduced sensitivity to thermal radiation of the heater, the maximum temperature of which could be 1200 K.

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Besides this, using the film dosimeter, a measurement of the electron beam AD on the shell surface (removed from the egg) and under the shell, as well as beneath the absorber layer (polyethylene of 80 microns thick), was performed (Figure 3). The sample was placed in a plastic container to preserve the geometry used for the irradiation of eggs.

The mathematically calculated practical range of electrons with a maximum energy of 500 keV in the shell of a chicken egg is ≈ 1.5-2.0 mm.

Assessment of NEB effect on salmonella cultures

The standard Salmonella genus microorganism strains were used. The seeding was carried out on a dense nutritional medium (Endo medium) at a dilution of 5 billion microbial cells per 1 ml. Immediately after seeding, the Petri dishes were subjected to a nanosecond electron beam with AD of 1, 2, 3 and 5 kGy. The experiments were carried out in 3 parallels, the control samples being present under the same conditions as the experimental ones, but without NEB treatment.

After irradiation, the dishes were placed in a thermostat and incubated for 24 hours at a temperature of 35 to 37ºC. An extreme multiplication rate is characteristic for bacteria. Division of a bacterial cell occurs every 20 to 30 minutes. Due to this, each bacterium can form an entire colony in 12 hours [11]. Thereby, at any high AD affecting the biological subject, a presence of one unit, cell or bacterium not exposed to the effect is possible. Therefore, the calculation of the amount of colony units in nutritional media (CFU) was carried out 24 hours after inhibiting.

For analysis of the biological effect, the logarithm of the number of formed colonies and the basic ballistic model accepted in radiobiology were used [12].

RESULTS

Ionization radiation sources and finding the absorbed dose

Using an accelerator URT-0.5 carried out the exposure experiments on eggs. In the first phase, the measurement of the AD distribution in depth in polyethylene (analog of biological tissue) was made by a gray wedge. During the experiments, the accelerator was operating at a charging voltage of 25 and 30 kV. Electron beam dosimetry results are shown in Figure 2.

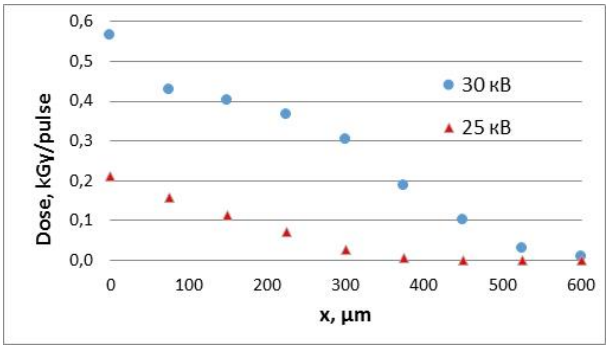

Fig 2: Distribution of the electron beam AD in the depth in the polyethylene at a different charging voltage on the accelerator URT-0.5.

The results of dosimetry using film dosimeter at a different charging voltage are shown in Table 1. However, AD under the shell with 20 and 25 kV was below the threshold of detector sensitivity, that did not allow to register the value.

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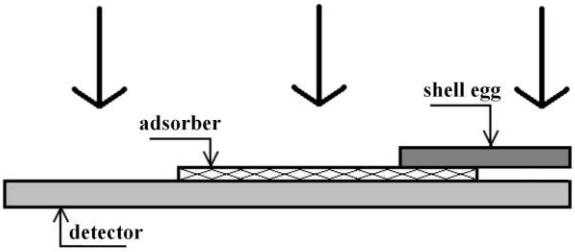

Fig 3: The geometry of the irradiation under the electron beam dosimetry.

The dosimeters were placed in sections of boiled eggs (cut lengthwise or crosswise), in such a way that it was possible to determine the AD distribution at various points of the biological object (Figure 4). The result of the assessment of the dose inside the egg is presented in Table 2.

Assessment of NEB effect on salmonella cultures

The biological response to the NEB is shown in Table 3. The number of colonies formed for all microorganisms' decreases with increasing AD. However, the radiosensitivity of biological objects is not the same. Respectively, the most sensitive bacterium to irradiation was Sal.ºTyphimurium and Sal. ºEnteritidis.

While Sal.ºGallinarum showed the greatest radioresistance and AD of 5ºkGy reduced the bacterial agglomeration by only two times. Therefore, large doses may be required for complete sterilization of bacteria. Figure 5 shows the presence of growth suppression at a dosage of over 1ºkGy in all microorganism strains.

When increasing the AD for Salt. Enteritidis, a practically linear increase in biological effect is observed. However, for Sal. Typhimurium and Sal.ºGallinarum, the NEB exposure effect does not increase proportionally to the dose. Irradiation of all samples with a dose of less than 3 kGy did not result in a decrease in the level of bacterial strains by more than 1 log.

In order to determine the radiobiological effect of ionization radiation on microorganisms, and in order to calculate lethal and semi-lethal doses of radiation, it is convenient to use dose-effect dependencies. Therefore, for the sake of simplicity, we used the exponential model:

$$S = e^{-a \cdot D}$$

where S is the number of cells relative to control sample in relative units, a is model coefficient, and D is the AD. This model is widely used in radiobiology [12].

The obtained dose-effect dependencies in Figures 6 allow the parameters of lethal LD37 and semi-lethal dose LD50 to be determined for each microorganism type – the values are outlined in table 4.

The obtained results are consistent with theoretical ones and with irradiation data from other papers

Table 1: Measurement results of the electron beam AD. *AD value is below the threshold of detector sensitivity

No.	Place of detector arrangement	AD, Gy/pulse at different charging voltages		
		30 kV	25 kV	20 kV
1	On the lid of a plastic container outside	583	505	173
2	On the surface of the shell	195	170	8
3	Under the shell	8.43	-*	-
4	Under the shell and absorber layer	0.61	-	-

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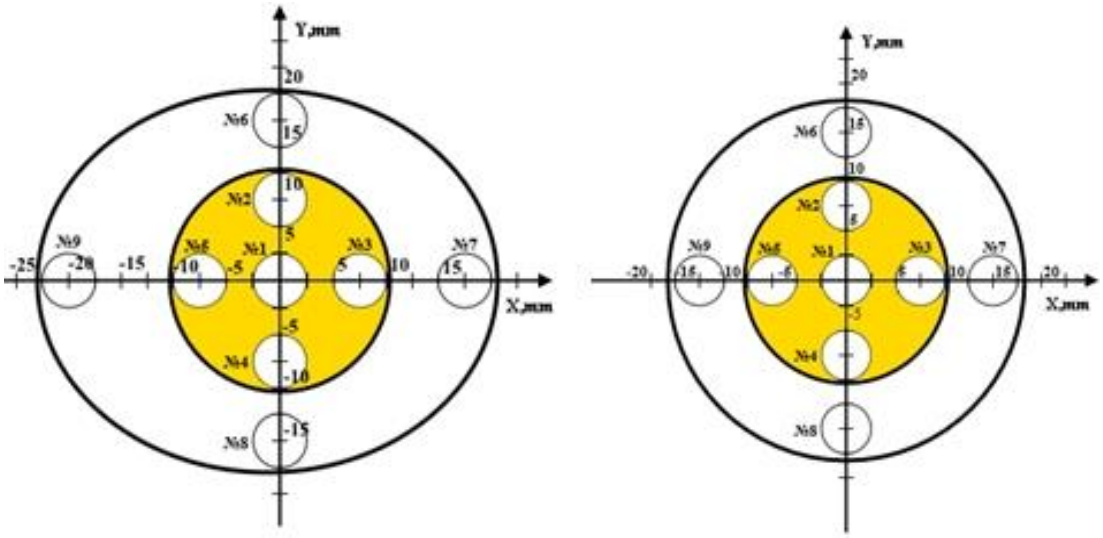

Fig 4: Location of TLD dosimeters (5 mm in diameter) at vertical (right) and the horizontal section of the hens' eggs.

Table 2: Measurement results of bremsstrahlung AD inside the egg.

Dosimeter No. (Figure 4)	AD, cGy/pulse	
	horizontal section	vertical section
1	0.13	0.16
2	0.25	0.15
3	0.18	0.17
4	0.17	0.13
5	0.15	0.14
6	0.18	0.18
7	0.31	0.21
8	0.15	0.15
9	0.17	0.26

Table 3: Microorganism survivability after exposure to NEB.

Microorganism	Dose, kGy (calculated)	Dose accounting for the pulse	Number of cells, CFU/g	LOG Number of cells, CFU/g
Sal. Enteritidis	0	0.0	1000	3.000
	1	1.5	500	2.699
	2	2.0	500	2.699
	3	3.0	250	2.398
	5	4.5	0	0.000
Sal. Typhimurium	0	0.0	1000	3.000
	1	0.5	400	2.602
	2	1.5	260	2.415
	3	3.0	250	2.398
	5	4.0	500	2.699
Sal. Gallinarum	0	0.0	1000	3.000
	1	0.5	500	2.699
	2	1.0	500	2.699
	3	3.0	100	2.000
	5	4.5	100	2.000

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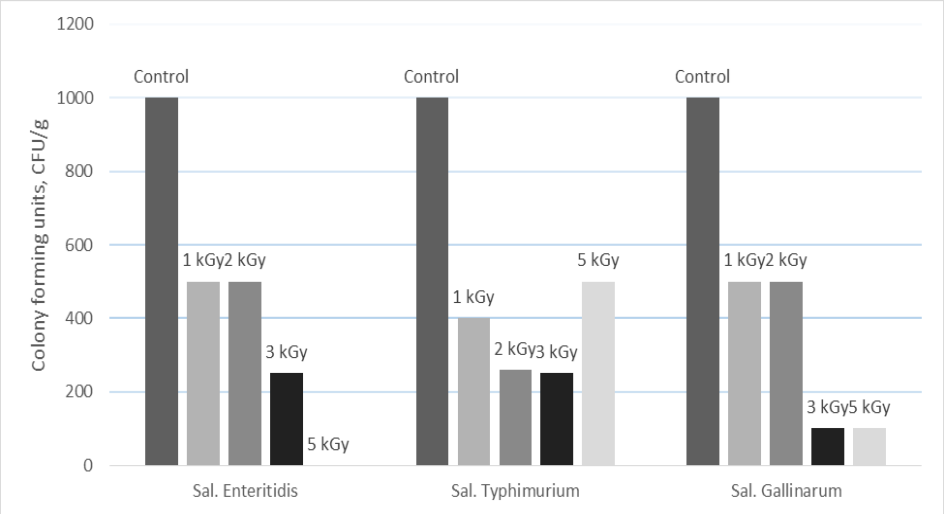

Fig 5: Microorganism survivability after NEB exposure: 1 – Sal. Enteritidis, 2 – Sal. Typhimurium, 3 – Sal. Gallinarum in 23 hours after exposure to the ionization radiation with AD of 1, 2, 3 and 5 kGy.

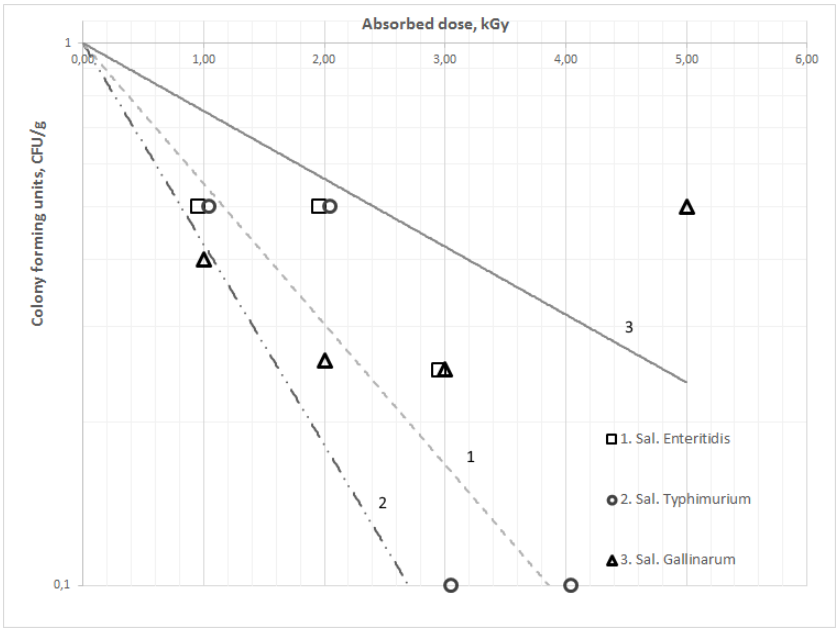

Fig 6: Dose-effect curves for microorganisms 1-Sal. Enteritidis, obtained using the exponential model.2 - Sal. Typhimurium, obtained using the exponential model. 3 - Sal. Gallinarum, obtained using the exponential model.

Table 4: Lethal dosages for microorganisms.

Microorganism strain	Sal. Enteritidis	Sal. Typhimurium	Sal. Gallinarum
LD50, kGy	0.813	1.163	2.407
LD37, kGy	1.172	1.675	3.472
LD05, kGy	3.512	5.026	10.402

The values of lethal dosages obtained using the standard radiobiological model do not indicate any difference of radioresistance in the three microorganism strains. At AD of 3 kGy and higher, significant suppression of growth of Sal. Enteritidis occurs. At AD of 5 kGy, the growth was fully suppressed.

Irradiation of the Sal. Enteritidis bacteria with radioactive 60Co isotope described in the paper [13] demonstrates an analogous biological effect at irradiation with AD of over 1.5 kGy. However, in one of the experiment series, the reduction in the number of the formed colonies reached 2 to 3 log with respect to the

control sample. The reaction of S. Typhimurium to gamma radiation led to a decrease of about 3 to 5 logarithms with respect to the control sample, with the irradiation doses within the range of 1.5 to 3.0 kGy [14].

DISCUSSION

As one can see from Figure 2, by varying the charging voltage of the accelerator, it is possible to choose the depth of electron penetration, in order to limit the exposure of the shell (0.3-0.4 mm) and the under-shell protein membranes (~70 µm) [5].

It should be noted that the shell consists of calcium carbonate with a density (2.74-2.83 g/cm3) and an atomic number close to those of aluminum. However, the shell is a porous structure and the passage of electrons through it will be a complicated process.

The data of Table 1 shows that at the charging voltage of 30 kV, it is possible to obtain the desired profile of the AD distribution at a depth at which the electron irradiation does not penetrate below the absorber layer simulating the under-shell egg membranes (Figure 3).

The measurement results showed that the bremsstrahlung AD inside the egg does not exceed 0.31 cGy/pulse, and at the yolk it is not more than 0.2 cGy/pulse (Table 2).

At the same time, the electron beam AD on the surface of eggs was 0.2 kGy/pulse (see Table 1). Consequently, at AD = 5 kGy, which can be produced by 25 pulses, and is sufficient to disinfect the surface of eggs from Salmonella, bremsstrahlung AD in protein will not exceed 8 cGy, and in yolk 5 cGy.

This AD value should not lead to biological transformations of the biological tissue, but rather should be within the AD range that has a stimulating effect on living organisms (radiation hormesis) [15].

Bremsstrahlung yield calculations under the irradiation surface of the egg (4.5 cm in diameter) by an electron beam from an accelerator URT-0.5 (electron current density per pulse ~ 3 A/cm2) were made according to Forster's formula [16] and in accordance with the biological protection calculation method for electron accelerators [17]. The results show that the AD is in the range of 0.11-0.15 cGy/pulse. Additional irradiation of the eggs is created by the electron beam bremsstrahlung being absorbed by the radiation-absorbing accelerator output structures, as well as by the scattered radiation.

In order to identify the differences of biological efficiency of NEB effect and gamma radiation, it is necessary to carry out studies under the same conditions of sample preparation and analysis. Due to this fact, alterations in the nutritional medium and radiation conditions may change the final result.

CONCLUSION

The results obtained lead to the conclusion that irradiation by an electron beam with AD level of 5 kGy is sufficient for complete disinfection on the surface of eggs. At the same time, the AD inside the egg will not exceed 8 cGy because of bremsstrahlung. This AD value should not lead to biological transformations of the protein or the yolk.

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