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Results Of Nanosecond Electron Beam Application For Antimicrobial Treatment Of Chicken Meat.

AS Krivonogova[1,3]*, AG Isaeva[3], KV Moiseeva[1], S Yu Sokovnin [1,2], IM Donnik[1], and PS Krivonogov[1].

[1] Ural State Agrarian University, Yekaterinburg, Russia
[2] Institute of Electrophysics UB RAS, Yekaterinburg, Russia
[3]Ural Research Veterinary Institute , Yekaterinburg, Russia

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-1 (electron energy up to 900 keV, pulse width about 90 ns, pulse repetition rate up to 300 pps). 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 meat surface. AD inside food product will not exceed 10 cGy due to bremsstrahlung. As well as mathematically calculated practical range of NEB shown that Track particles will not exceed a depth of 2-3 mm Histological and physicochemical analysis did no show significant influence on meat. Therefore, irradiation of meat with NEB allows sterilization without changes in product quality.
Keywords: *Disinfection, nanosecond electrons beam, bremsstrahlung, dosimetry, sterilization, broiler meat,*

Corresponding author

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.

In raw chicken meat, there is a high probability of the presence of bacteria dangerous to the human organism, such as Salmonella, Listeria, Escherichia coli and Campylobacter. The most effective method for controlling pathogenic bacteria and microorganisms is treating chicken meat with radiation. Treatment of the meat with radiation allows for the meat shelf life to be prolonged 2 to 3-fold [1]. However, surface pasteurization or sterilization using high temperature (~100ºC) is a more expensive procedure and can reduce product quality.
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 [2].

The irradiation of the foods could be accompanied by a variety of chemical reactions that may transform the organoleptic properties of the products and decrease quality 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 [3]. 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 approach.

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 doesn't significant influence on the inside of the food product.

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.

At present, nanosecond electronic accelerators for technologies have been developed and manufactured [4], 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 [5]. 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, that is a positive properties. Unfortunately, completely avoid the irradiation of meat interior part is impossible, since bremsstrahlung is induced by absorption of the electrodes, which makes a major contribution to the AD created inside the meat.

MATERIALS AND METHODS

Ionization radiation sources and dosimetry control

AD was tested by a film dosimeter CO AD (F) R-5/50 [6] 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.

To determine the distribution of AD bremsstrahlung inside meat, 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 [7]. 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.

Besides this, using the film dosimeter, a measurement of the electron beam AD on the surface meat and under meat layers, as well as beneath the absorber layer (polyethylene of 600 microns thick), was performed..

The exposure experiments on meat were carried out by a pulsed repetitive nanosecond accelerator URT-1 [8] (electron energy up to 900 keV, pulse width about 90 ns, pulse repetition rate up to 300 pps).

Because of the low electron energy (less than 1 MeV), the practical range is not deep and AD distribution will be inhomogeneous. However, bremsstrahlung will influence on the entire biological object. Thus, the AD will be the maximum on length of the track of electrons and will be the minimum in the remaining volume of the samples.

The practical range of electrons with a maximum energy of 900 keV in meat is ≈ 2 - 3 mm.

Histological assay of broiler meat treated with NEB

In order to study the alteration in the meat structure after NEB irradiation, we carried out a histological study of broiler chicken fillet. 30 measurements (15x2 parallels) were made for histological analysis. The fixation was carried out with Eosin using formalin with further paraffin molding. Staining with hematoxylin Eosin was carried out according to Boehmer. All samples were split into 5 groups: “experiment 1” were subjected to irradiation with AD of 2 kGy; “experiment 2” with 5 kGy; “experiment 3” with 10 kGy and the control groups “control 1” and “control 2”.

The dose range selected in our study is determined by standard irradiation doses for meat sterilization. The broiler chicken meat exposed to ionization radiation treatment at a dosage of 2.5ºkGy and 6 kGy and stored at a temperature of 0ºC to +2ºC has the parameter of number of mesophilic aerobic and facultatively anaerobic microorganism of 4.7-5.0×10 CFU/g to 2.6-4.2×10^3 CFU/g, and the frozen meat at a temperature of -18ºC – from 4.4-4.5×10 CFU/g to 1.2-1.4×10^2 CFU/g correspondingly, which is compliant with the requirements of SanPiN 2.3.2.1078-01 [1].

Physical and chemical study of broiler meat after NEB treatment

In order to study the alteration in general physical and chemical properties, the samples of lumpy semi-finished meat products from broiler chickens were subjected to NED exposure with the AD of 0, 2, 5 and 10 kGy. After the exposure, the samples were subjected to standard testing methods in order to determine physical and chemical properties. pH was measured by the difference in electrical potentials between the glass electrodes and the reference electrolyte placed in the sample [GOST R 51478-99].

Moisture weight percentage was determined by the method of drying the analyzed sample with sand to a constant mass at a temperature of 103º±º2º°C [GOST 33319-2015]. To determine the protein and nitrogen weight percentage we used method based on the mineralization of the Kjeldahl test and the photometric measurement of the intensity of the indophenol blue color [GOST 25011-81]. Fat weight percentage was measured by repeatedly extracting the fat with a solvent from the dried sample analyzed in the Soxhlet extraction apparatus, followed by removing the solvent and drying the isolated fat to a constant weight [GOST 23042-2015].

Spectrophotometric method for determining the mass fraction was used to determine the phosphorus weight percentage [GOST 32009-2013]. The method is based on drying the sample, calcining the residue, followed by cooling and hydrolysis of the ash with nitric acid, filtration, dilution of the filtrate with a mixture of ammonium mononadate and ammonium molybdate hept to form a yellow compound. After that, a photometric measurement of the optical density is carried out at a wavelength of 430 nm.

The method used to determine the weight percentage of chlorides is based on the precipitation of proteins and the titration of excess silver nitrate solution with potassium thiocyanate solution in an acidic medium in the presence of iron ammonium alum as an indicator [GOST R 51480-99].

Measurements of the mass fraction of amino acids by the method of high-performance liquid chromatography were used to study the chemical properties of irradiated meat. [M-02-902-142-07].

RESULTS

Ionization radiation sources and finding the absorbed dose

Using an accelerator URT-1 carried out the exposure experiments on the meat.

The dosimeters were placed in sections of meat, in such a way that it was possible to determine the AD distribution at various points of the biological object. The measurement results showed that the bremsstrahlung AD inside the meat does not exceed 0.2 cGy/pulse at distance up to 5 cm in depth of a piece of meat.

Histological study of broiler meat treated with NEB

In the samples from control and 1st experimental groups, the muscular fibers are mostly unchanged, transversely striated lineolation is clearly manifested, there are separate unformed muscular fibers, and non-uniform distribution of protein in separate muscular fibers. In the “experiment 2” group (NEB AD of 5 kGy), features of protein coagulation in muscular cells and distortion of transversely striated lineolation were found. In the “experiment 3” group (NEB AD of 10 kGy), features of protein fibrillation, distortion of cellular sarcoplasm structure, lineolation distortion, protein coagulation and also sarcolemma distortion were found.

Table 1. Physical and chemical parameters of the broiler meat after NEB treatment.

Parameter	**Unit**	**NEB dose (kGy)**			
		0 Gy (control)	2 kGy	5 kGy	10 kGy
Hydrogen parameter	pH	5.9	5.9	5.7	6.0
Moisture weight percentage	%	75.85	75.05	75.40	76.15
Protein weight percentage	%	22.75	22.47	22.60	22.68
Nitrogen	%	3.64	3.60	3.62	3.63
Fat weight percentage	%	1.70	1.80	2.20*	2.00*
Phosphorus weight percentage based on P2O5	%	0.40	0.37	0.38	0.40
Phosphorus weight percentage	%	0.18	0.16	0.16	0.17
Chlorides	%	Less than 1	Less than 1	Less than 1	Less than 1
AMINO ACIDS					
Parameter	**Unit**	**NEB dose (kGy)**			
		0 Gy (control)	2 kGy	5 kGy	10 kGy
Glutamic acid	%	3.61	3.56	2.70	2.43
Oxyproline	%	0.049	0.077	0.056	0.044
Serine	%	0.82	0.86	0.66	0.60
Glycine	%	0.93	1.00	0.73	0.69
Histidine	%	0.52	0.48	0.38	0.37
Arginine	%	1.32	1.20	0.99	0.83
Threonine	%	1.08	0.73	0.74	0.63

Alanine	%	0.90	0.65	0.60	0.51
Tyrosine	%	0.75	0.69	0.57	0.52
Valine	%	0.97	0.77	0.73	0.68
Lysine	%	2.11	1.95	1.71	1.60
Tryptophan	%	0.174	0.206	0.118	0.189
Cysteine	%	0.12	0.13	0.13	0.070
Methionine	%	0.59	0.51	0.34	0.37

Therefore, in the meat samples treated with NEB with AD of 5 kGy and higher, there is damage to cellular proteins, which is manifested by myfibrillae separation, lineolation distortion, fiber fibrillation and also distortion in cellular membrane, sarcolemma, structure. Alteration in cellular membrane structure can lead to alteration in the membrane permeability, which should affect biochemical processes in muscular fiber. Decrease in peroxide lipid oxidation is apparently associated with alteration in membrane permeability, which positively affects the storage of frozen meat.

Moreover, features of protein damage in surface layers of muscular cells indirectly indicate the fact that at the meat surface the NEB dosage providing for protein damage has been reached that causes damage to protein in bacterial cells, which substantially confirms the antibacterial effect of the NEB.

The organoleptic parameters of broiler chicken meat in the paper [1] exposed to the dosages of ionization radiation of 2.6 and 6.0 kGy do not differ from the meat of control chickens. The body surface of the experimental groups has a white and pink color, the muscles are elastic, of average moisture at cutting, internal fat is of yellowish color. The broiler chicken meat irradiated with 30 kGy had an odor manifested in deviation determined by fat rancidity.

The effective electron run distributes AD from the NEB non-uniformly in biological sample, and the main AD is distributed at the depth of 2 mm. The AD provided by penetrating impact radiation does not exceed dozens of cGy in the entire volume of the broiler chicken fillet. Such dosage cannot lead to protein fibrillation, distortion in cellular sarcoplasm structure, distortion of lineolation, protein coagulation or sarcolemma distortion. Therefore, there are no significant negative factors inside the fillet.

As a whole, these alterations in the surface meat layers exposed to NEB over 5 kGy should not adversely affect the production quality, because penetration depth of the electrons, and correspondingly the depth of exposed tissues, is quite low.

Treatment of broiler chicken meat with ionization radiation at dosages of 2.5; 6.0 and 30.0 kGy leads to a weakly manifested reduction in the biological value of the meat in uptake and safety: in experiments on laboratory animals, the OBC is reduced by 0.5%, which confirms the safety of the irradiated meat for consumers. Therefore, in order to increase shelf life of the cooled and frozen meat of broiler chickens, it is expedient to carry out sterilization with ionization radiation at dosages of 2.5 and 6.0 kGy [1].

Physical and chemical parameters of broiler meat after NEB treatment

On investigating the physical and chemical parameters of the chicken meat, no significant alterations were found in experimental samples compared to control ones. Nor were any trends of alteration of parameters upwards or downwards at increase of the AD of less than 2 kGy found. In treatment of semi-finished products with AD of more than 5 kGy, an increase of the fat weight percentage is observed.

Data outlined in Table 1 reflect a pronounced trend towards a dose-dependent decrease in the weight percentage of separate amino acids. Thus, average parameters in the samples treated with NEB with AD of 10 kGy were less than in control samples: for glutamic acid the decrease was 33%, for serine 27%, for histidine 29%, for arginine 37%, for threonine 42%, for alanine 43%, for tyrosine 31%, for valine 30%, for lysine 24% and for methionine 38%. The obtained results correspond to the results obtained in the paper [2]. However, time dependence should be taken into account. In treatment of the meat with ionization radiation with the dosages of 2.5 kGy, 6 kGy and 30 kGy, a decrease in the pH parameter occurs: 1.3-fold in average in 5 days; 1.5-fold in 1.5 and 6 months correspondingly.

DISCUSSION

The obtained results indicate that irradiation of broiler meat with ionization radiation has a sterilization effect, thus safeguarding the biological value of the food product. In view of the low electron run, the main dosage load is distributed at a depth of 2 mm. The remaining part of the meat is subjected to impact radiation only. This means that the AD from bremsstrahlung is significantly less and does not exceed several dozen cGy. This AD cannot lead to significant alterations of broiler meat. Also, the surface of the meat is fully sterilized, reducing microbiological contamination.

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 poultry meat. At the same time, the AD inside the meat lumps will not exceed 10 cGy because of bremsstrahlung. This AD value should not lead to biological transformations of the protein, the yolk, or the poultry meat.

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