Investigation of microbial death kinetics during relativistic electron beam processing for Salmonella enterica as an example

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Abstract. While processing plant raw materials, it is necessary to observe the balance of effective dose and quality for processed products. The solution to this problem may provide an adequate direction to determine the optimal processing criteria. For define the minimum processing criteria, information database is required, which makes it possible to determine the mechanisms of processing effect appearing. This base is also a key element in the construction of experimental work that allows improving the technology of applying physical methods in the food industry. The present work objective is to study the consistent patterns of pathogenic microorganisms’ inhibition depending on the electron beam energy and the accumulated dose on Salmonella enterica as an example. As the object of research the museum strain of Salmonella enterica subsp. enterica serovar Typhimurium ATSS 140283 cultivated on an artificial model medium was used. The results of the research showed a two-phase nature of the microorganisms’ death kinetics. This work is based on microorganisms’ death kinetics research. The models for the response of two subpopulations stable and unstable for relativistic electron beam processing have been developed. The distribution of the processing hardness with a subpopulation that is unstable to relativistic electron beam processing was established. The need to combine processing by a relativistic electron beam with other types of active exposure to achieve the maximum sterility rate for the entire population of microorganisms has been experimentally established.

1. Introduction

Processing of raw materials and finished products by physical methods is a promising economical and functional solution to the problem of quality and safety [1, 2]. Even though research in this area has been continuing worldwide for many years [1, 3, 4], many unexplored issues need to be settled, especially about changes in the quality characteristics of plant raw materials during processing and storage. One of the main criteria for the treatment effectiveness is an achievement or required degree of pathogenic and opportunistic microflora inactivation.

Investigations of antiseptic efficiency for various physical methods have shown [5] that microorganisms have different degrees of susceptibility regardless of the type of treatment. Works
devoted to the study of microorganisms inhibition on the surface of model media under the influence of ionization radiation show [5, 6] that Salmonella subspecies are ones of the most stable type of microorganisms. In many works, it was noted that while staying liquid or solid media within various installations death kinetic of treated microorganisms demonstrated the plateau zone existence during a middle range of used dose values [5, 6]. There are also differences in processing efficiency in plants with different capacities. Researches in this area have not been fully carried out. They are of interest under the point of view as reducing the total burden of physical processing the plant raw materials.

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During explaining the effects of ionization radiation on microbial cells, there is no common imagination for mechanisms leading to the realization of the initial damage for the structural components of living systems to final visible effects. Solving this problem requires an enhanced analysis of the processes occurring in a living system at each stage of the radiation energy exchange, describing the whole bunch of molecular changes, and creating a model that captures the entire sequence of physical and chemical processes.

Per the present work, the objective is to study the consistent pattern of pathogenic microorganisms’ inhibition depending on the electron beam energy and the accumulated dose on *Salmonella enterica* as an example.

Research purposes are to obtain experimental data and their statistical processing in a wide range of electron beam energies and accumulated doses, development of the primary and secondary models of the microflora death kinetics.

2. Materials and methods

As the object of research the museum strain of *Salmonella enterica* subsp. enteric serovar Typhimurium ATSS 140283 (State Research Center for Applied Microbiology & Biotechnology, Russia), cultivated on an artificial model medium was used.

As a model growth medium, we used a dense nutrient medium (meat-peptone agar – MPA) which has the basic set of nutrients and contributes to the maintenance of viable microorganisms during the research process.

The suspension of microorganisms was obtained by culturing the museum strain in a liquid nutrient meat-peptone broth (HiMedia Laboratories Pvt. Limited, India) by cultivation at a temperature of 37 °C for 24 hours. Then the suspension was placed to Petri dishes on the surface of the MPA based on the concentration of microorganisms as ~10^8 CFU/g.

Petri dishes were placed in sealed cases made with BOPP polymer film with a thickness as 40 microns. The samples were processed using a linear electron accelerator UELR-10-15-C-60-1 (Tecleor LLC, Russia) with relativistic electrons beam with energy as 5, 6, 7, 8 and 9 MeV till reaching the accumulated dose 0,8, 1,3, 1,7, 2,2, 2,4, 3,0, 3,4, 3,8, 4,0, 4,2, 4,8 and 5.0 kGy.

The processed samples were placed in a thermostat to determine the residual concentration of the surviving microflora. Initial samples were used as controls.

Cultivation was performed at temperature and exposure, according to [7, 8].

The values of the residual concentration of the surviving microflora were determined based on the results of counting viable colony-forming microorganisms. The microorganism concentrations were calculated as a weighted average from the data of two consecutive dilutions:

\[
N = \frac{\sum C_i}{V \cdot 1.1 \cdot d}
\]

where \( N \) is the concentration of microorganisms, CFU/g; \( \sum C_i \) is the sum of the colonies counted in Petri dishes within two consecutive dilutions, CFU; \( V \) is the volume of suspension introduced into each Petri dish, cm³; \( d \) is the dilution coefficient corresponding to the first (smaller) selected dilution.
The calculation results were rounded to two significant digits.

Mathematical processing of experimental data was carried out using specialized software Table Curve 2D v.5.01 (SYSTAT Software Inc.), Wolfram Mathematica 10.4 (Wolfram Research Inc.) and table processor Microsoft Excel 2010 (Microsoft Corporation).

3. Discussion of the results

Visual analysis of experimental data in the system "dose – microorganisms concentration" showed that in this coordinate system, the data could be successfully interpreted based on a subpopulation approach, in which the microbial death kinetics as a processing result is a superposition of the death kinetics for two subpopulations with difference only in the resistance degree.

In this regard, the experimental data were approximated to determine the dose-concentration relationships using the two-phase Coroller model [9], which is a superposition of two Weibull models of death kinetics [10].

\[
\log N = \log N_0 + \log \left( f \cdot 10^{\frac{D}{\delta_1}} + (1 - f) \cdot 10^{\frac{D}{\delta_2}} \right) 
\]

(2)

where \( N_0 \) – the initial concentration of microorganisms, CFU/g; \( f \) – is the fraction of the first subpopulation in the initial concentration of microorganisms, in-unit shares; \( \delta_1 \) – the dose required to reduce the concentration of the first subpopulation for the first order, kGy; \( \delta_2 \) – the dose required to reduce the concentration of the second subpopulation for the first order, kGy; \( p_1 \) and \( p_2 \) – power indices, respectively, for the first and the second subpopulations; and \( D \) is the accumulated dose, kGy.

Due to the proximity of the \( f \) value to unit, Geeraerd proposed to transform this value to optimize the calculations [11]:

\[
\alpha = \log \left( \frac{10^\alpha}{1+10^\alpha} \right) 
\]

(3)

where \( \alpha \) is an empirical power exponent.

In this case, the original model may be easily decomposed into two components corresponding to the death kinetics of each subpopulation:

\[
\log N_I = \log \left( N_0 \cdot \frac{10^\alpha}{1+10^\alpha} \right) - \left( \frac{D}{\delta_1} \right)^{p_1} 
\]

(4)

\[
\log N_{II} = \log \left( \frac{N_0}{1+10^\alpha} \right) - \left( \frac{D}{\delta_2} \right)^{p_2} 
\]

(5)

where \( N_I \) and \( N_{II} \) are the microorganisms’ concentration of subpopulations I and II, respectively, CFU/g.

The values of the main indicators of the death kinetics models corresponding to the experimentally set electron energy are presented in Table 1.

| Indicators | 5 | 6 | 7 | 8 | 9 |
|------------|---|---|---|---|---|
| Model      | \( \alpha \) | 2.247 | 3.715 | 4.324 | 2.698 | 4.324 |
|            | \( R^2 \)   | 0.988 | 0.953 | 0.998 | 0.999 | 0.972 |
| I subpopulation | \( \delta_1 \) | 0.360 | 0.556 | 0.725 | 0.739 | 0.525 |
|            | \( p_1 \)   | 1.011 | 1.157 | 4.811 | 3.959 | 3.279 |
| II subpopulation | \( \delta_2 \) | 2.795 | 3.641 | 3.335 | 2.692 | 3.097 |
|            | \( p_2 \)   | 5.859 | 6.471 | 7.963 | 2.749 | 8.83  |

Table 1. Main indicators of models of the kinetics of microbial death (\( p<0.05 \))
A particular result of approximating the microbial death kinetics as a result of processing with a relativistic electron beam is shown in Figure 1. We analyze the array of data obtained as a result of primary modelling. Based on this analysis, it can be seen that the ratio of each indicator can be traced some dependence on the value of the electron energy at which the processing was performed. Each indicator is an independent variable in the mathematical description of the models.

In this regard, approximation functions describing these dependencies were obtained:

$$\alpha = \exp \left( a + b \cdot E + c \cdot E^2 + d \cdot E^3 \right)$$ \hspace{1cm} (6)

$$\delta_1 = \exp \left( a + b \cdot E + c \cdot E^2 + d \cdot E^3 + e \cdot E^4 \right)$$ \hspace{1cm} (7)

$$p_1 = a + b \cdot \exp \left( -\exp \left( \frac{E - d \cdot \ln (\ln 2) - c}{d} \right) \right),$$ \hspace{1cm} (8)

$$\delta_2 = \exp \left( a + b \cdot \exp (-E) \right)$$ \hspace{1cm} (9)

$$p_2 = a + b \cdot E^2 + c \cdot E^4 + d \cdot E^6$$ \hspace{1cm} (10)

where $E$ is the electron energy, MeV.

These functions have a pronounced clear empirical (fitting) character; so further analysis of their physical content does not make much sense. However, in the field of determining experimental values of electron energy, these functions adequately describe the demanded dependencies with $p \leq 0.005$.

**Table 2. Coefficients and statistical characteristics of empirical dependencies described by formulas (6)-(10)**

| Indicators | $R^2$ | Coefficients |
|------------|-------|---------------|
|            | a     | b             | c     | d    | e   |
| $\alpha$   | 1.00  | 13.815        | -5.977 | 0.909 | -0.044 | -- |
| $\delta_1$ | 1.00  | 13.815        | -10.46 | 2.582 | -0.264 | 0.0097 |
| $p_1$      | 0.95  | 0.986         | 0.715  | 6.448 | 1.028 | -- |
| $\delta_2$ | 1.00  | 1.162         | 12.653 | --   | --   | -- |
| $p_2$      | 1.00  | 0.999         | 0.409  | -0.011 | 1.3·10^{-4} | -- |
The coefficients and statistical characteristics of the established dependencies are presented in Table 2. Based on the approximation results, a secondary (three-dimensional) model of the microbial death kinetics as a result of processing with a relativistic electron beam was obtained (Figure 2).

![Figure 2. 3D model of the microbial death kinetics (spheres – experimental points; blue ones – ones laying on the response surface; red ones – below the surface; green ones – above the surface)](image)

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Analysis of primary models of the microbial death kinetics in the dose – concentration coordinates showed that there is a certain range of doses between the value of the accumulated dose corresponding to a significant sterilizing effect (6 or more orders of magnitude) for an unstable (first) subpopulation and that for the beginning of noticeable microbial death in a stable (second) subpopulation, during which the processing doesn’t have any significant effect on the superposition of concentrations. We name it “a sensitivity pause.” The existence of the sensitivity pause confirms the insufficiency of processing only relativistic electrons to achieve an acceptable sterility rate for the entire culture and, accordingly, the need to create conditions for suppressing a stable subpopulation by other physical methods.

Analysis of existing concepts of factors that are significant in the processing of relativistic electron beam has shown that in addition to the value of the accumulated dose, the rate of the dose set can also be attributed to such factors, the variation of which in the simplest version can be set by changing the energy of the electrons. In order to take this factor into account, the second model was presented in the two-phase form (Figure 3), taking into account the separate contribution of each of the two subpopulations.

Visual analysis of this model shows the presence of a sensitivity pause in almost the entire area of determining the experimental values of electron energy.

The nature of the response surfaces of the second model allows stating that the rate of dose set, determined by the electron energy, does affect the processing efficiency with an unstable subpopulation. Besides, the initial calculated concentration of microorganisms in a stable subpopulation decreases with the increasing rate of dose set, which generally demonstrates an increase in the overall destructive effect. For almost the entire range of variation of experimental electron energy values to achieve a sterility rate as 8 orders of concentration for an unstable subpopulation, the sufficient value of the accumulated dose does not exceed 2.5 kGy at minimum energy values, and ~2 kG y or less at maximum ones.
Figure 3. The two-phase version of the 3D model of the microbial death kinetics (the painted surface is the response for an unstable subpopulation; the unpainted surface is the response for a stable subpopulation).

However, different combinations of electron energy and accumulated dose correspond to different degrees of processing hardness. At the same time, a priori, among the appropriate processing options, the optimal one is one in which the processing hardness for the object being processed will be minimal. In this regard, based on the three-dimensional death kinetics of the unstable subpopulation the processing hardness palette was calculated for the entire region of determining for the values of the sterility rate and the electron energy (Figure 4) using the formula:

$$J(E, n) = E \cdot D = E \cdot f_{\delta_1}(E) \cdot n^{1/p_1(E)}$$

(11)

where $J(E, n)$ – processing hardness, MeV·kGy; $f_{\delta_1}(E)$ – functional dependence $\delta_1$ on $E$, determined by the formula (6); $n$ – sterility rate, concentration orders; $f_{p_1}(E)$ – functional dependence $p_1$ on $E$, determined by the formula (8).

Figure 4. Influence of the electron energy and the achieved sterility rate on the processing hardness.
The analysis of the obtained results showed that in order to achieve a sterility rate for the unstable subpopulation as up to 6 or more orders of concentration while processed by relativistic electron beam, several values of electron energy simultaneously correspond to the minimum processing hardness. In this case, the strength of this dependence is determined by the desired value of the sterility rate. However, postulating that the minimum energy consumption for processing corresponds to the minimum of the given electron energy, the value of 5 MeV was taken as an optimal value of the electron energy.

4. Conclusion
Thus, the following results were obtained during the research:
- it was experimentally established the two-phase nature of the microbial death kinetics of the Salmonella subspecies as a result of processing by the relativistic electron beam in the energy range from 5 to 9 MeV;
- based on the modelling of microbial death kinetics, response models for stable and unstable subpopulations for processing by relativistic electron beam have been developed;
- the distribution of the processing hardness with a subpopulation that is unstable to relativistic electron beam processing was established;
- the need to combine processing by a relativistic electron beam with other types of active exposure to achieve the maximum sterility rate for the entire population of microorganisms has been experimentally established.

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