Influence of parameters of omelets' treatment process with high pressure on their microbiological safety

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Abstract: *The article contains the results of researches on dependencies of omelets microbiological contamination (E. coli, Pseudomonas fluorecens, Paenibacillus polymyxa and Listeria seeligeri) with various fillers on the parameters of their high pressure treatment process (HP) (pressure value, temperature and process duration). The fact that it is reasonable to use kinetic models of the second order to describe the process of colon bacillus (Escherichia coli) inactivation is proposed for the first time and is experimentally established. Dependences of changes in the constants of inactivation rate ln (k1) and ln (k2) on the pressure for kinetic models of the second order were obtained in research. In the article were obtained values of omelet microbial contamination, treated by HP E. coli, Pseudomonas fluorecens, Paenibacillus polymyxa and Listeria seeligeri at their long storage.*

Key Words: omelets with fillers, storage period, microbiological safety, high pressure, temperature.

I. Introduce

Hen eggs are one of the most valuable human food products and are used in the preparation of a large number of dishes, among which the leading place is occupied by omelets. Unfortunately, this product is not intended for long-term storage and it is prepared at the public catering facilities as needed. At the same time, taking into account its high nutritional value, this product [1,2], provided that its high nutritional and consumer properties are ensured at a long-term storage period, can be recommended for use in expeditions and tourist trips, hard-to-reach regions of the country, at the formation of strategic reserves of the armed forces and Navy, as well as at the eggbreaking plants, food industry and public catering facilities.

The most appropriate for the development of production process of mixed omelets with various fillers of long-term storage period is to use the HP technology, which provides their microbiological safety of various food products [3-5] during the storage while preserving the entire enzymic and vitamin complex. In addition to the sterilizing effect high pressure [6-9] has positive effect on various physical and chemical properties of liquid egg [10], which significantly increases both the scientific and practical value of researches on the effects of HP on egg and omelets with different fillers.

Authors of numerous researches have studied the influence of HP on various representatives of microbial flora both liquid egg in whole and its separate components [11-22]. Researches that consider the inactivation of microorganisms by HP in complex matrices [21] are of interest, as well as the study of the synergetics of the combined effects of HP, ultrasound and other factors on the processed product [22-24]. Unfortunately, there are almost no researches that studied the effects of HP on omelets with different fillers.

The aim of the research: is to determine the dependence of indicators of microbiological safety in the process of long-term storage of omelets with various fillers on parameters of its high pressure treatment process.

The object of research is high pressure treatment process of omelets with various fillers.

The subject of research – the technological parameters of high pressure treatment process and microbial indicators of omelets with various fillers.

II.Methodology of research

In order to study the influence of HP on egg products on the basis of liquid hen egg there was developed the process of producing omelets with cheese, bacon and fried champignons of long-term storage. This process lies in mixing liquid egg with grated or finely chopped cheese (or other ingredients), xanthan gum, which gives the form-

stable ability to the finished product, then adding water or milk, spices (salt, pepper), and then the obtained mixture is packed in a sealed elastic packing material, it is heated, then it is put into the working chamber with HP installation. The obtained product in the sealed package is intended for long-term storage, and that's why the study of its microbiological safety during the storage is the priority while determining the rational parameters of both the process of its production and the storage modes of the produced product.

The treatment of omelet samples was conducted at a high-pressure unit (HPU) [25] in the range of process parameters: mixture preheating at the 85-95^{0}C, pressure 650-750 MPa, treatment time -up to 8 min.

As a result of the fact that the product that is loaded into the working chamber of HP has a temperature of 85-95^{0}C, and the subsequent increase in pressure in the working chamber, the temperature, at which carried out the high pressure treatment process was equal to 110-130^{0}C.

In order to perform the experimental studies on evaluation of microbiological sterility of food samples treated with HP, there was used E. coli culture, which was prepared as follows.

E. coli, which was discovered in one of the samples of liquid hen eggs and relegated to the group K12DH5α, was carried to 20ml of standard broth and cultivated in vibrating incubator during 24 hours at 30^{0}C. After 24 hours of incubation, 50ml of the suspension was carried to 20ml of fresh broth and the cultivation continued for another 24 hours. Subsequently, 1ml of suspension of microorganisms with E. coli were grafted to 100ml of a whole egg, which was used in the production of omelet. The initial concentration of E. coli was approximately 108 CFU / ml. The initial concentration of other microorganisms used in microbiological studies was approximately 107 CFU / ml.

The main pathogenic microorganisms found out in pasteurized liquid eggs are: Alcaligenes, Bacillus, Proteus, Escherichia coli, Pseudomonas and Gram-positive cocci.

The procedure of experimental researches on the study of influence of parameters of omelet high pressure treatment process consisted of the following stages:

1. Formation of a Bank of microbiological cultures for their subsequent introduction into omelet samples;
2. Preparation of omelet samples with cheese, bacon and champignons according to the technology and introduction of long-prepared microbiological culture;
3. Omelet samples packaging in sterile sealed containers and their treatment at HPU;
4. Microbiological analysis of omelet samples, both directly after the HP treatment, and during their long-term storage at +4±0,5^{0}C.

Microbiological analysis of omelet samples treated by HP, was performed using the standard methods according to ISO-4833: 2003 IDT (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony-count technique at 30 degrees C), ISO-21528-2004 (ISO 21528-1:2004 «Microbiology of food and animal feeding stuffs -- Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 1: Detection and enumeration by MPN technique with pre-enrichment), ISO 4833:1991 «Microbiology - General guidance for the enumeration of micro-organisms - Colony count technique at 30 degrees C», ISO-6579:2002-07, GOST 10444.15-94 «Food Products. Methods for determination the quantity of mesophilic aerobic and facultative anaerobic microorganisms» and GOST 10444.12-88 «Food products. Method for determining yeast and fungi», «Unified sanitary and epidemiological and hygienic requirements for goods that are subject sanitary and epidemiological surveillance (control) ».

Taking into account the fact that in omelet production are used several ingredients, including milk that has not undergone the thermal sterilization, there were analyzed safety requirements for each component of omelet.

In addition to mentioned above indicators, there was studied the influence of HP on three species of psychrophilic bacteria: Listeria seeligeri (Listeria innocua), Pseudomonas fluorecens, Paenibacillus polymyxa, which are often the cause of food spoilage during their storage in refrigerated condition [1]. The indicator "Coliform bacteria" is selected in accordance with the accepted international nomenclature, it is almost identical to the indicator of "Coliforming bacteria". During the study were taken into account both citrate-negative and citrate-positive variants of Coliform bacteria, including the following genus – Escherichia, Klebsiella, Enterobacter, Citrobacter и Serratia.

All of the mentioned above culture samples were obtained as a result of inoculation and subsequent dilution of microbial flora samples to the required concentration, detected and identified during various microbiological analyses.

II. Materials and methods

Inactivation of microorganisms under HP, as well as denaturation of proteins, is often described by kinetic equations of the first order, as a result of which the logarithm of concentration of

microorganisms that survived after pressure treatment decreases linearly with the increase in treatment time t as $– kt$, where k is the constant of inactivation rate.

$$-\frac{dN}{dt} = k \cdot N \qquad (1)$$

where N is the number of viable organisms; k is the constant of inactivation rate.

Integral equation (1) with taking into account initial conditions, $N=N_0$ in $t=0$ was presented as:

$$\ln(\frac{N}{N_0}) = -k \cdot t \qquad (2)$$

Equation (2) offers linear dependence of N on t on a semi-logarithmic scale and was expressed using common logarithm:

$$\ln(\frac{N}{N_0}) = 2.303 \cdot \log(\frac{N}{N_0}) \qquad (3)$$

The constant of inactivation rate (k) is the most commonly used concept to describe the thermal inactivation of microorganisms.

In literature, there are often data with significant deviations from linearity, which are usually described by a combination of two reactions of first-order as two-phase kinetics with different inactivation rates [19,20]. Two-phase kinetics is common for both vegetative and spore forms of bacteria. In such cases, there is a retardation in the fall of the logarithm of concentration over time, the rate of fall for small and large t is equal to k_1 and k_2, respectively, and, most often, $k_1 > k_2$. Such form of inactivation curve indicates the existence of a small part of population the increased resistance to HP effect.

Similar studies were carried out for omelet samples with bacon and champignons, as a result of which were determined the values of parameters of omelet treatment process with high pressure, providing microbiological safety of the product at long storage:

Analysis of experimental data on inactivation of Escherichia coli have shown that in order to describe the kinetics of inactivation of E. coli at the given process parameters, it is reasonable to apply two-phase model of the first order. This model consists of two parts that follow the independent kinetics of first order (Fig. 1).

The surviving microorganisms during t are the sum of separate parts:

$$N(t) = N_1(\tau) + N_2(\tau) \qquad (4)$$

The analytical solution of the mentioned above equation is presented as:

$$N(t) = N_0(f \cdot e^{-k_1 \cdot t} + (1 - f) \cdot e^{-k_2 \cdot t}) \qquad (5)$$

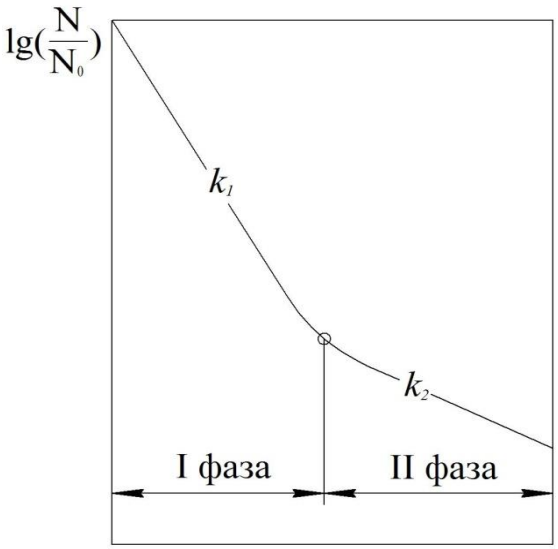

Figure 1.. *Typical curve of two-phase inactivation*

where N_0 is the initial number of microorganisms and f is the initial proportion of the first part (N_{01}/N_0).

Each part of this inactivation model is expressed as:

$$\frac{dN_1}{dt} = -k_1 \cdot N_1(t), \; N_1(0)=N_{01} \qquad (6)$$

$$\frac{dN_2}{dt} = -k_2 \cdot N_2(t), \; N_2(0)=N_{02} \qquad (7)$$

where N_1 and N_2 are the number of microorganisms in the first and second parts, τ is the treatment time;

k_1 and k_2 are the constant of inactivation rate.

Dependence of the constants of inactivation rate on the pressure was analyzed by the Arhenius-type model. Dependence of pressure and the constant of inactivation rate k is described by the following equation (8):

$$\left(\frac{\partial \ln k}{\partial P}\right)_T = -\Delta V^* / RT$$

where k is the constant of inactivation rate of first order in s^{-1}, P is the pressure in MPa, ΔV^* is the apparent volume of activation in m^3 $mole^{-1}$, R is the gas constant $8{,}314 \times 10^{-6} \cdot m^3 \cdot$* MPa $mole^{-1} \cdot K^{-1}$, T is the temperature in Kelvin degrees, K.

Equation (8) shows that the dependence of ln (k) on pressure at the constant temperature is described by inclined line $-\Delta V^*/RT$.

The program STATISTICA V5.5A was used for kinetic analysis of Escherichia coli inactivation process.

Due to the fact that kinetic model of the second order can't be adequately evaluated by the linear model, there was performed the nonlinear estimation using piecewise linear regression and were obtained the values of points of discontinuity of the second-order curves (table. 2).

As a result of statistical analysis of experimental data the E. coli inactivation process was described by the following function for all values of its parameters

$$\begin{cases} y = a + c \cdot (x - b) & x < b \\ y = a + d \cdot (x - b) & x > b \end{cases} \quad (9)$$

III. Results and discussion

The numerical values of the model coefficients for different values of process parameters and statistical characteristics of these dependencies are represented at the Table 1. The confidence interval is 0.95.

Figure 2 shows the experimental points and the piecewise linear dependencies of decrease in the relative concentration of E. coli in the samples of omelet with cheese.

Table 1 *Results of experimental data statistical processing*

Pressure MPa	*a*	*c*	*d*	*b* point of discontinuity	R^2	F stat	Str. err
Temperature 110^0C							
650	-3,005	-0,0121	-0,0074	240	0,987	188,9	0,185
700	-1,877	0,0152	-0,0108	120	0,99	772,1	0,106
750	-4,476	0,0224	-0,0101	180	0,94	39,5	0,93
Temperature 120^0C							
650	-4,341	-0,0183	-0,012	240	0,99	319,94	0,219
700	-3,498	-0,0304	-0,015	210	0,99	624,38	0,19
750	-4,157	-0,0346	-0,013	120	0,99	732,61	0,17
Temperature 130^0C							
650	-6,913	-0,0223	-0,0053	300	0,99	315,94	0,262
700	-6,543	-0,0255	-0,0085	240	0,99	249,24	0,303
750	-6,092	-0,0296	-0,0093	180	0,96	162,72	0,579

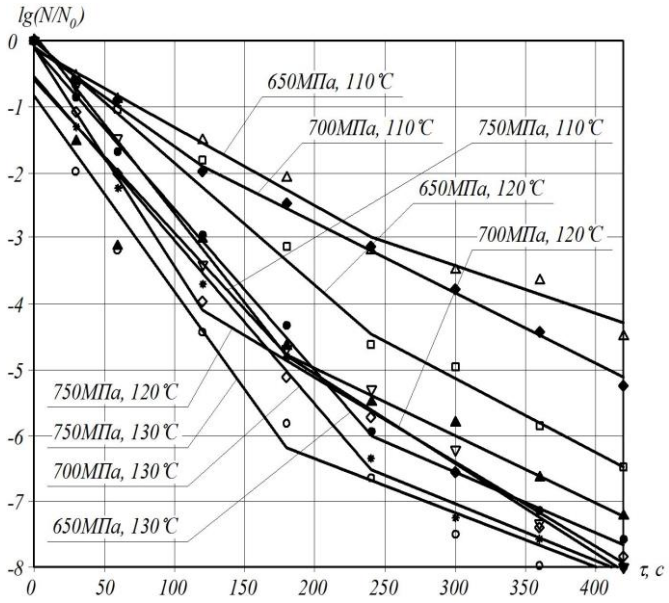

Figure 2. The experimental points and the piecewise linear dependencies of decrease in the relative concentration of E. coli in the samples of omelet with cheese

Mathematical description of E. coli inactivation process at different values of process parameters allowed to obtain and analyze the dependences of constants of inactivation rate ln (k_1) and ln (k_2) on pressure for the functions described by kinetic models of the second order.

Graphical interpretation of dependence of the reaction rate constant value on the process parameters (pressure and temperature) is presented in Fig. 3 and it allowed to analyze the dynamics of reaction rate constant at different phases of the process.

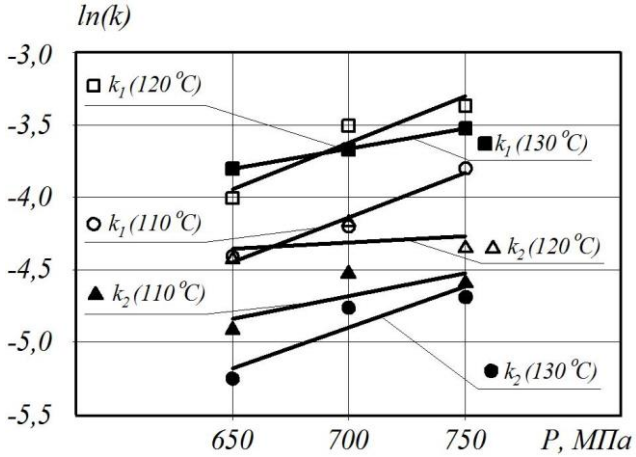

Figure. 3. Dependence of constants of inactivation rate ln (k_1) and ln (k_2) on the pressure

Table 2 shows the results of experimental researches on the influence of parameters of omelet treatment process with high pressure on provision of microbiological sterility in respect of such

microorganisms as mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM); pathogenic microorganisms, including Salmonella; psychrophilic bacteria *Listeria seeligeri (Listeria innocua), Pseudomonas fluorecens, Paenibacillus polymyxa*.

Due to analysis of presented results it can be stated that these process parameters allow to provide the necessary level of microbiological safety of the product.

Table 2 *Values of microbial contamination of omelet samples at different values of parameters of treatment process*

Process parameters			Coliform bacteria in 1,0g	pathogenic, incl. Salmonella in 25g.	*S.aureus* in 1,0g	*Proteus* in 0,1g	QMAFAnM CFU/g
temperature, ^{0}C	pressure MPa	Treatment products, s					
Omelet with cheese							
121	700	420	n/d	n/d	n/d	n/d	n/d
Omelet with bacon							
121	700	420	n/d	n/d	n/d	n/d	n/d
Omelet with champignons							
121	700	420	n/d	n/d	n/d	n/d	n/d

- * n/d-not detected

Thus, for the first time we obtained functional dependences of changes in the relative concentration of Escherichia coli at the omelet treatment with HP at different process parameters. For the first time there was experimentally established and explained the fact that at different parameters of the treatment process it is reasonable to use kinetic models of the second order. Dependences between the changes in the constants of inactivation rate ln (k_1) and ln (k_2) and the pressure for kinetic models of the second order were obtained in research. In the article were obtained values of omelet microbial contamination with *E. coli* depending on parameters of omelet high pressure treatment process and here also were determined the technological parameters of the process, providing microbiological safety of omelet against *Pseudomonas fluorecens, Paenibacillus polymyxa and Listeria seeligeri*.

To study the dynamics of indicators of microbiological safety of omelet samples with cheese, bacon and champignons, treated by HP in the process of its storage were used the samples, produced with the process parameters: 700MPa – 121 ^{0}C - 6 min. Studied samples were stored in the sealed packaging, in which they were treated with HP at the temperature 4±0.5 ^{0}C with the relative humidity from 85% to 88%. Replication of measurements at this point is three times. Microbiological parameters were monitored each 30 days of storage.

Analysis of microbiological safety by 5 previously mentioned indicators showed that during 6 months of storage in omelet samples were not detected: Coliform bacterias in 1.0 g, pathogenic, including *E. coli* in 25g., *S.aureus* in 1.0 g, *Proteus* in 0.1 g. At the 5-th and 6-th months of storage were detected QMAFAnM (Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms) in the amount of $1x10^5$ and $1x10^4$ CFU/g, which is significantly lower than permissible values for this indicator. Psychrophilic bacteria of the species *Listeria seeligeri (Listeria innocua), Pseudomonas fluorecens и Paenibacillus polymyxa* also were not detected.

IV. Conclusions

The conducted researches allowed to determine the dependence of omelet microbial contamination (*E. coli, Pseudomonas fluorecens, Paenibacillus polymyxa и Listeria seeligeri*) on the parameters of their high pressure treatment process (pressure value, temperature and process duration). The fact that it is reasonable to use kinetic models of the second order to describe the process of colon bacillus (Escherichia coli) inactivation is proposed for the first time and is experimentally established. Dependences of changes in the constants of inactivation rate ln (k_1) and ln (k_2) on the pressure for kinetic models of the second order were obtained in research. In the article were obtained values of omelet microbial contamination with different fillers (cheese, bacon, fried champignons), treated by HP *E. coli, Pseudomonas fluorecens, Paenibacillus polymyxa* и *Listeria seeligeri* at their long storage.

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