Proving the Pasteurization Mode of the Canned Fish Using culture of Enterococcus Faecium

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Abstract This article considers the questions of developing the pasteurization modes of the canned foods of D group which is an actual direction in the modern food technology. The culture of Enterococcus faecium has been chosen in quantity of 1·10⁷ CFU/g. The possibility of using these microorganisms as a test culture during scientific proving the developed mode of pasteurization of canned foods called “Trout in olive oil with the addition of pickled pineapple” has been proven in this article. The test results have proven the microbiological safety of used semi-product (fish and canning mass) before pasteurization: QMAFAnM is 6.2·10³ CFU/g for trout and 9.0·10³ for canning mass; pathogenic and conditionally pathogenic microorganisms haven’t been found. The thermostability constant of D have been determined experimentally by heating the culture of Enterococcus faecium in capillaries at the temperature of 80 – 95 °С during the prefixed time followed by calculating the number of survived cells and data processing using graphical method and least square method. The normative pasteurization effect has been calculated using previously determined thermostability constants, it is of 52.9 conditional minutes. This value has been used for determining the optimal duration (60 minutes) of pasteurization process of canned foods at the temperature of 85 °С. The effective and scientifically proved pasteurization mode of canned foods of D group called “Trout in olive oil with the addition of pickled pineapple” has been chosen.

1. Introduction
There is a scientific approach to develop and prove the new canned foods sterilization modes, requiring the complex of microbiological, physical and chemical, thermophysical, and technological researches. Using of non-proved sterilization modes can result in such unacceptable defects of canned foods as swell, flipper, microbiological spoilage etc. which can be dangerous for consumers’ life and health or can result in essential losses of the finished product [1].

The practical lethality (L), which must be higher than normative (Fн), is considered as a primary characteristic during developing the sterilization modes [2], [3]. There is a table of normative sterilization effects for the main assortment of the sterilized canned fish in “Instruction for developing sterilization modes for fish and marine products” (1996, in Russian); it is recommended to use these values as basic during developing the new kinds of canned foods. But there is no such normative information about pasteurized canned fish which can be classified as D-group [4].

The aim of current research is to determine the normative effect of pasteurization developed by authors [5] of new kind of delicacy canned fish of D group called “Trout in olive oil with the addition of pickled pineapple” using test culture of Enterococcus faecium.

The following tasks have been solved to achieve this aim:
- to determine the thermostability constants of test culture of Enterococcus faecium;
to calculate the normative pasteurization effect for canned food, and comparing it with practical, to choose thermal and temporal parameters of pasteurization (pasteurization mode) of canned foods.

2. Materials and methods

2.1. Research materials
The chilled trout (fitting the Russian standard GOST 814) has been used as the raw materials for producing the pasteurized canned foods. The pickled pineapple and the olive oil have been used as flavoring agents. The test culture of Enterococcus faecium has been used for developing the normative pasteurization effect.

2.2. Methods of analysis
Determining the normative sterilizing effect has been carried out according the common method in a several stages based on the capillary tube method by Stern and Proctor (1954). The advantage of this method is the possibility of processing of 10 and more samples simultaneously [6], [7]. This method was chosen as a basis during developing the pasteurization modes of canned foods of D group.

The culture of Enterococcus faecium have been chosen as a test culture for determining the normative pasteurization effect. Enterococcus faecium is a species of enterococci which is included in the composition of normal microflora of the human (and some other Mammals) intestine. Enterococci are the gram-positive bacteria which are neither spore-forming nor capsule-forming, they are facultative anaerobes (can use the fermentation energy, and so they can live both at high and very small amount of oxygen).

Using the Enterococcus faecium test culture in quantity of $1 \times 10^7$ CFU/g (which is enough to result in the product spoilage) allows to determine an optimal duration of the thermal processing which is enough to kill both the vegetative cells (completely) and the spore-forming bacteria (the most of them: acceptable residual quantity is not higher than $2 \times 10^2$ CFU/g) in the canned fish. Restricting the percentage of defective product samples from 0.1 to 0.01 % provide obtaining canned foods more stable in storage.

Determining the thermostability constants of the test culture of Enterococcus faecium has been carried out by testing surviving the microorganisms by counting live cells after heating the aliquot of the test culture in the glass capillary tubes during the fixed time in the thermostat at the temperatures of 80 – 85 – 90 – 95°C.

The chilled trout has been tested for presence of conditionally pathogenic (coliforms, Staphylococcus aureus, Vibrio parahaemolyticus) and pathogenic (Salmonella spp., Listeria monocytogenes) microorganisms; the quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFA,nM) has also been determined using the standard methods [8], [9].

The quantity of spores of mesophilic clostridiums and thermophilic bacilli has been additionally determined according to the “Instructions for sanitary and microbiological control of food production from fish and marine invertebrates” (1991). Data processing has been carried according to ISO 7218 “Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations” using MPN (most probable number) method [10]. The identification of microorganisms has been carried out by cultural, morphological, physiological, and biochemical features using Bergey's Manual of Systematic Bacteriology [8], [11].

3. Results and discussion

3.1. Testing the microbial safety of the raw materials
The series of tests has been carried out to determine the sanitary and microbiological characteristics of the canned foods before pasteurization [12]. The results are shown in Tables 1 and 2.

Data of Table 1 shows that the chilled trout fit the normative documentation – the Technical Regulations of the Eurasian Economic Union #040/2016 [4].
Table 1. Results of microbiological safety tests of the chilled trout

| No. | Characteristic, unit | Normative value [4] | Test result |
|-----|---------------------|---------------------|-------------|
| 1.  | Mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), CFU in 1.0 g, not higher | $1.0 \times 10^3$ | $6.2 \times 10^3$ |
| 2.  | Coliforms, in 0.001 g | Not acceptable | Not found |
| 3.  | *Staphylococcus aureus*, in 0.01 g | Not acceptable | Not found |
| 4.  | *L. monocytogenes*, in 25 g | Not acceptable | Not found |
| 5.  | *Salmonella* spp., in 25 g | Not acceptable | Not found |
| 6.  | *V. parahaemolyticus*, CFU in 1.0 g, not higher | 100 | Less than 10 |
| 7.  | Sulfite-reducing clostridia, in 1.0 g | - | Not found |
| 8.  | Spore-forming mesophilic aerobic and facultative anaerobic microorganisms of the group of *B. cereus* in 1.0 g | - | Not found |

The additional studies (besides required according the regulations) have been carried out by the following characteristics: “Sulfite-reducing clostridia, in 1.0 g” and “Spore-forming mesophilic aerobic and facultative anaerobic microorganisms of the group of *B. cereus* in 1.0 g” for the purpose of eliminating the presence of such microorganisms in the pasteurized canned foods [8], [13]; the results are shown in the Table 2.

Results of Table 2 can make the conclusions that the microbiological characteristics of the canned foods are not exceed the normative values [8], [14].

Table 2. Results of microbiological safety tests of canning mass in the can before pasteurization

| No. | Characteristic, unit | Test result |
|-----|---------------------|-------------|
| 1.  | Mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), CFU in 1.0 g | $9.0 \times 10^3$ |
| 2.  | Coliforms, in 0.001 g | Not found |
| 3.  | *Staphylococcus aureus*, in 0.01 g | Not found |
| 4.  | Sulfite-reducing clostridia, in 1.0 g | Not found |
| 5.  | *Salmonella* spp., in 25 g | Not found |
| 6.  | Spore-forming mesophilic aerobic and facultative anaerobic microorganisms of the group of *B. cereus* in 1.0 g | Not found |

Two new kinds of canned foods [5], [15] “Trout in olive oil with the addition of pickled pineapple” in tin cans № 2 (net mass of product is 170 g) have been developed on the department of Food Production Technology. They can be classified as the canned foods of D-group (pasteurized canned foods) [4]. Canned foods have been produced according to four pasteurization modes with the
temperature interval from 80 to 95 °C and the constant duration of the process of 60 minutes (Table 3). The practical lethality has been experimentally determined for each pasteurization mode using temperature sensors TrackSense Pro (Ellab, Denmark).

| Table 3. Pasteurization modes |
|-----------------------------|
| Pasteurization temperature, °C |
| 80  | 85  | 90  | 95  |
| Duration of the process, min |
| 60  | 60  | 60  | 60  |
| Practical lethality, cond. min |
| 52.8| 99.7| 254.9| 468.2|

Determining the normative pasteurization effect was necessary to choose the most acceptable for these four pasteurization modes.

The thermostability constants of cells of test culture of Enterococcus faecium have been studied to determine the normative pasteurization effect for canned foods “Trout in olive oil with the addition of pickled pineapple”.

3.2. Determining the thermostability constant D

The culture of Enterococcus faecium was heated in the capillary tubes at the temperatures from 80 to 95 °C during specified time. After heating it was seeded to the BMEH agar (agar for the cultivation of microorganisms based on beef meat enzymatic hydrolysate) and to the lactose-cystine agar “Uriselect” [16], then it was thermostated (1-2 days, 37 °C). Then the number of survived cells has been calculated.

The experimental results (4 replicates) are shown in Table 4.

| Table 4. Number of survived cells of Enterococcus faecium at the different duration of the heating |
|-------------------------------------------------------------|
| Heating duration, min | Temperature, °C | Number of colonies grown on the culture medium, CFU/g | Decimal logarithm of average survived cells number |
|------------------------|-----------------|-----------------------------------------------|--------------------------|
| First repetition        | Second repetition | Third repetition | Fourth repetition | Average value |
| 0.25                   | 85              | 9.0·10⁶         | 9.1·10⁶         | 8.9·10⁶       | 9.1·10⁶       | 9.0·10⁶       | 6.950        |
| 0.5                    | 2.2·10⁴         | 2.1·10⁶         | 2.3·10⁶         | 2.2·10⁶       | 2.2·10⁶       | 2.2·10⁶       | 6.342        |
| 0.75                   | 1.7·10⁷         | 1.7·10³         | 1.6·10⁵         | 1.7·10⁵       | 1.7·10⁵       | 1.7·10⁵       | 5.230        |
| 1.0                    | 1.4·10⁷         | 1.3·10⁴         | 1.5·10⁴         | 1.3·10⁴       | 1.3·10⁴       | 1.3·10⁴       | 4.146        |
| 1.5                    | 2.2·10⁷         | 2.1·10³         | 2.3·10⁵         | 2.1·10⁵       | 2.2·10⁵       | 2.2·10⁵       | 3.342        |
| 2.0                    | 1.0·10⁷         | 1.1·10⁴         | 1.1·10⁴         | 1.0·10⁴       | 1.0·10⁴       | 1.0·10⁴       | 3            |
| 2.5                    | 5.5·10⁷         | 5.6·10⁴         | 5.4·10⁵         | 5.5·10⁵       | 5.5·10⁵       | 5.5·10⁵       | 2.740        |
| 3.0                    | 2.7·10⁷         | 2.6·10²         | 2.8·10²         | 2.8·10²       | 2.7·10²       | 2.7·10²       | 2.431        |
| 4.0                    | 35              | 34              | 36              | 35            | 35            | 1.544        |
| 5.0                    | 1               | 0               | 1               | 1             | 1             | 1             | 0.954        |
| 6.0                    | 4               | 3               | 4               | 4             | 4             | 4             | 0.602        |
| 8.0                    | 2               | 1               | 3               | 2             | 2             | 2             | 0.3          |
| 10.0                   | 0               | 0               | 1               | 0             | 0             | -             |

The microorganisms surviving curve has been drawn after determining the thermostability of cells in the researched product (Fig. 1).
Figure 1. Enterococcus faecium cells survival curve at different duration of capillary tubes heating

The equation of $y=a+bx$ (where $x$ – duration, sec., $y$ – decimal logarithm of survived cells number) has been obtained on processing the data of Enterococcus faecium cells surviving at the different heating duration [10]. Using the regression analysis by the least square method ($a=7.186$; $b=−0.0395$) the straight line corresponding the surviving of Enterococcus faecium has been drawn (Fig.2).

![Figure 1](image1.png)

Figure 2. Plot for determining the D constant

The D constant has been calculated based of the obtained dependency using the following way. The surviving plot (Fig.2) has been used to find value of $D_T$. To do this, the straight lines have been drawn
from points corresponding one logarithmic cycle (for example, 1 and 2) to crossing with the obtained straight line. The founded crossing points have been projected to abscissa axis. The difference of them (2.09 – 1.7 = 0.39 = 0.4) is the value of D_1.

Thus, the carried-out experiments of studying of thermostability of test culture Enterococcus faecium followed by calculations have resulted in determining the constant a D = 0.4 min.

3.3 Determining the duration of the pasteurization

The normative pasteurizing effect (F_n) has been calculated using obtained thermostability constants of D and Z with the following formula [7]:

\[ F_n = D \times (Lg \left( \frac{C_0 \times V \times 10^{100}}{S} \right) + x) \times k, \text{ cond. min}, \]

where:
- \( D \) – duration of heating the product at the constant temperature, which results in decreasing the microorganisms’ number 10 times;
- \( T_0 \) – standard temperature (85 ºC);
- \( C_0 \) – number of inoculated test-microorganisms before pasteurization, cells in 1 g of product (1\times10^7 cells/g);
- \( V \) – the volume of the can, cm\(^3\);
- \( S \) – percentage of defective cans (0.1 %);
- \( x \) – correction for surviving of vegetative microorganisms after pasteurization (2);
- \( k = 10 \) – conversion factor chosen according to the recommendations "Guidelines for the development of science-based sterilization and pasteurization of canned food and canned semi-finished products" [17].

\[ F_n = 0.4 \left( Lg \left( \frac{1 \times 10^7 \times 170 \times 100}{0.01} \right) + 2 \right) 10 = 52,921 \text{ cond. min}. \]

Normative pasteurizing effect is 52, 9 conditional minutes.

Basing on the calculated result, the conditions of pasteurization has been chosen (Table 3) to fit the inequality of \( L \geq F \). The practical lethality of them is 99.7 cond. min. Thus, the duration of the pasteurization of the current canned foods at the temperature of 85 ºС is defined to be 60 min, so, the effective pasteurization mode for canned foods of D-group “Trout in olive oil with the addition of pickled pineapple” in cans № 2 (net mass 170 g) is determined:

\[ 15 – 60 – 20 \]
\[ 85^\circ C, \text{ L= 99.7 cond. min}. \]

Pasteurization followed by chilling must be done using water in vertical autoclaves of periodic action.

Conclusions

The possibility of using the culture of Enterococcus faecium as the test culture during developing the pasteurization modes of canned foods development has been proved.

The thermostability constant of D for the test culture have been experimentally determined; the normative pasteurization effect has been calculated for the D-group canned fish “Trout in olive oil with the addition of pickled pineapple”.

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