Розроблено режими пост-пастеризації цільном'язових делікатесних м'ясних продуктів, які дозволяють істотно подовжити термін зберігання без змін органолептичних показників виробу.

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Досліджено вплив короткочасного термічного оброблення готового упакованого під вакуум делікатесного м'ясного продукту на мікробіологічні, фізико-хімічні та органолептичні показники.

На основі моделювання температури і тривалості оброблення розроблені раціональні режими пост-пастеризації, які максимально збільшують термін придатності зберігаючи високу якість продукту.

Отримано залежності впливу пост-пастеризації на мікробіологічні характеристики та доведено, що розроблені режими ефективно пригнічують поверхневу мікробіоту.

Дослідження по визначенню раціональних режимів пост-пастеризації проводили у діапазоні температур 75–90 °С та тривалості 1–4 хв.

Використання пост-пастеризації протягом 1-2 хв. при температурі 90 °С, а також протягом 3 хв. при температурі 75–85 °С викликає зменшення кількості бактеріального забруднення, але термін придатності продукту не збільшується. Оброблення при 90 °С протягом 4 хв. суттєво зменшує кількість мікроорганізмів, подовжую термін придатності, але спостерігаються зміни зовнішнього вигляду продукту.

Визначено, що раціональними режимами пост-пастеризації є температура 90 °С та тривалість 3 хв. Представлено дослідження бактеріологічних ефектів, які досягненні використанням пост-пастеризації при даних режимах.

Проведено дослідження впливу режимів пост-пастеризації на ключові показники якості готового продукту. Встановлено, що наведений спосіб температурного оброблення не викликає синерезису вільної вологи, що має важливе значення для вакуумної упаковким'ясних продуктів.

Враховуючи, що рН впливає на ріст мікробіоти, проведено дослідження концентрації водневих іонів безпосередньо після пост-пастеризації та в процесі зберігання.

Доведено, що використання розроблених режимів пост-пастеризації дає можливість подовжити термін придатності цільном'язових делікатесних м'ясних виробів у вакуумній упаковці на 10 діб

Ключові слова: пост-пастеризація, термічне оброблення, мікробіота, делікатесні м'ясні продукти, термін зберігання

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# 1. Introduction

Stable production of high-quality meat and meat products is one of the most important requirements of the meat industry. Demand for high-quality and safe products subjected to minimum processing and having long shelf life is steadily increasing. Food safety is a top priority for consumers worldwide.

Microbiological contamination is the most important factor that determines safety of a meat product and limits

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# **ESTABLISHING** TEMPERATURE AND **TIME FACTORS** FOR THE POST-PASTEURIZATION **OF GOURMET MEAT** PRODUCTS

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its shelf life [1, 2]. Microorganisms can enter the product at any stage of the production process where their development depends on their species, the product and environmental conditions determining reproducibility of microorganisms [3–6].

When a meat product gets ready-to-eat after thermal treatment, it has an insignificant number of microorganisms [7, 8]. As regards mortality of the microbiota, thermal treatment used in the production of meat products is effective but it cannot prevent recontamination during its cutting and packaging [9, 10].

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The ready-to-eat meat product manufactured with proper processing without violating sanitary regime and storage conditions is highly stable. Microbiology related problems appear because of non-compliance with specified conditions of thermal treatment and storage [11]. Insufficient thermal treatment can lead to survival of heat-resistant strains of *Lactobacillus and Enterococcus* [12].

The products packed after the boiling stage, are more prone to bacterial contamination. Contamination is mainly caused by growth of *Lactobacillus and Carnobacterium* which produce lactic acid and impart a sour-sweet odor [13].

Lactic acid bacteria form the major group of bacteria that cause spoilage of meat products including whole-muscle meat products after their thermal treatment. Their number grows because of combination of microaerophilic conditions in the product created by sodium chloride and sodium nitrite and a decrease in water activity. These factors inhibit growth of other microbial groups and provide an advantage for lactic acid bacteria to multiply [14, 15].

Thermal processing of meat is considered to be the most widespread technology of microbiota destruction. It is used separately or in combination with other new technologies of processing raw meat materials [16]. Various methods and conditions of thermal processing are used to preserve meat and prepare new products [9, 17, 18].

Thermal treatment at a temperature of up to 100 °C kills all vegetative cells: *E. coli, Pr. vulgarus*, lactic acid bacteria, yeast, and other saprophytic non-spore-forming microorganisms. At the beginning of the process, tens of thousands of microbial cells are contained in 1 kg of raw material. In a meat product that is brought to a condition of culinary readiness by means of high temperature, there are up to hundreds of cells [2, 9, 19].

Whole-muscle gourmet meat products are made from the parts of slaughtered animal carcass subjected to salting and thermal treatment to the ready-to-eat condition [20]. These products are characterized by excellent taste, high nutritional value and are in high demand among consumers [12]. The whole-muscle meat products differ from other meat products in that they retain cellular structure of meat components during the production process, up to consumption [21].

The problem of re-contamination of gourmet meat products is extremely important as they are the non-encapsulated products mostly eaten without additional thermal treatment.

One way to solve the problem of re-contamination of gourmet meat products may consist in developing such conditions of thermal treatment of the ready-to-eat products which will ensure not only extension of their shelf life but also high quality.

Therefore, the study of establishment of temperature and time factors of post-pasteurization to extend shelf life and improve quality of gourmet meat products is relevant.

#### 2. Literature review and problem statement

Thermal treatment of ready-to-eat meat products is used to inactivate microorganisms, extend the product shelf life as well as create a possibility of storing meat products even at temperatures above 4 °C [22]. In-package pasteurization of ready-to-eat products can be the final stage in destroying vegetative microbiota and be an effective method of preventing product spoilage [23]. Effect of thermal treatment and high pressure on readyto-eat boiled sausages for inhibiting microbiota and extension of the product shelf life were studied in [24]. Samples of boiled sausages in a vacuum package were kept in a water bath at 80 °C for 15 min. after temperature in the product reached 75 °C. High pressure treatment was carried out at 600 MPa. Studies have shown that pasteurization and highpressure processing can be used to increase the product safety and extend its shelf life. Studies have also shown that pasteurization is more effective than high-pressure treatment.

The results of studies in [25] demonstrate effectiveness of using thermal treatment to extend shelf life of Viennese sausages. For example, to extend shelf life up to 4 times, thermal treatment of ready-to-eat vacuum-packed sausages at a temperature of 80 °C for 20 min. was used. Studies on the use of thermal treatment of ready-to-eat sausage products were also carried out in [26]. The study results indicate effectiveness of pasteurization in relation to microbiological safety.

Possibility of extending shelf life of boiled sausages using repeated pasteurization was studied in [27]. To extend shelf life of boiled small sausages up to 72 days, repeated pasteurization at a temperature of 85–90 °C for 15–20 minutes was used.

The use of pasteurization of cooked chicken thighs individually packed in a vacuum bag was effective to reduce number of microorganisms including *Listeria innocua* [28].

In [29], vacuum packed sausages heat treated to a core temperature of 90 °C and 100 °C were edible for 9 days at a storage temperature of 31 °C.

Experiments with small sausages which were grilled, vacuum packed and then heated at 95 °C for 5 min. in a steam chamber were conducted in [30]. Repeated thermal treatment of packaged products substantially extended their shelf life. However, thermal treatment of sliced sausage in these conditions has destroyed the product structure.

Appearance of samples of baked and vacuum-packed turkey heated at 95  $^{\circ}$ C has changed, however the shelf life was 4 weeks longer than that of the control sample.

Studies of pasteurization of a ready-to-eat product that were carried out with samples having protective covering (encapsulation) have shown no organoleptic changes. However, there is a significant problem of repeated processing non-encapsulated meat products because of possibility of alteration of their appearance, structural and qualitative characteristics under long influence of heat [31].

It is clear from the literature analysis that the issue of thermal treatment of non-encapsulated whole-muscle meat gournet has not been resolved.

## 3. The aim and objectives of the study

The study objective is to develop conditions of post-pasteurization to extend shelf life of gourmet meat products without worsening their quality.

To achieve this objective, the following tasks were set:

 to experimentally determine rational temperature and time conditions of post-pasteurization;

- to determine safety and quality of the product after post-pasteurization based on microbiological, physicalchemical and organoleptic characteristics;

 $-\,{\rm to}\,$  establish a rational shelf life of gourmet meat products after post-pasteurization based on comprehensive studies.

# 4. Materials and methods used in studying the effect of post-pasteurization conditions on a gourmet meat product

To extend shelf life of gourmet meat products by post-pasteurization, samples were taken from premium Balyk Marochny smoked boiled meat produced by Garmash PE (Odessa oblast, Ukraine) according to TU U 15.1-33381354-007:2012 Boiled, Boiled and Smoked, Boiled and Baked, Fried, Raw-smoked, Raw-dried Products of Pork, Beef, Horse Meat and Poultry. Specifications.

The following post-pasteurization parameters were chosen to determine duration of thermal treatment:

- sample 1.1: *t*=90 °C, τ=1 min;
- sample 1.2: t=90 °C,  $\tau=2$  min;
- sample 1.3: *t*=90 °C, τ=3 min;
- sample 1.4: t=90 °C,  $\tau=4$  min.

To determine optimal post-pasteurization temperature, studies were conducted with the following thermal treatment parameters:

- sample 2.1:  $\tau$ =3 min, t=75 °C;
- sample 2.2:  $\tau$ =3 min, t=80 °C;
- sample 2.3:  $\tau$ =3 min, t=85 °C;
- sample 2.4:  $\tau = 3 \min, t = 90 \degree C.$

Microbiological, physicochemical, and organoleptic studies of the control and test samples were performed on the first day of storage as well as on day 14, 21, 28, 35 and 42 of storage.

More detailed study materials and methods are described in [32].

## 5. The results obtained in the study of effect of postpasteurization conditions on the gourmet meat product

Microbiological characteristics were studied in the process of studying duration of post-pasteurization at a fixed temperature of 90  $^{\circ}$ C. The results obtained are presented in Table 1.

The study results presented in Table 1 indicate that the use of thermal treatment of packaged gourmet meat products at 90 °C is effective for a significant reduction of number of the microorganisms.

The total amount of bacterial contamination on day 28 indicates that the control sample as well as samples 1. 1 and 1. 2 were inedible during storage and exceeded the MAOAnM normative value of  $10^3$  CFU/g.

Samples 1.3 and 1.4 had a tolerable number of color ny-forming units (CFU) for the entire storage period of 35 days, did not exceed the MAFanM normative value and were edible.

Taking into account the results presented in Table 1, it can be stated that duration of treatment of samples 1. 1 and 1. 2 was insufficient to significantly reduce degree of microbial contamination. Also, these samples had the same shelf life as the samples that did not undergo post-pasteurization.

Table 2 presents results of determination of hydrogen ion concentration (pH) in the control and test samples at all storage stages.

Data in Table 2 show that no significant changes in concentration of hydrogen ions occur after post-pasteurization. pH in the control sample increased during the storage process more rapidly than in the samples subjected to thermal treatment.

| Tabl | e 1 |
|------|-----|
|------|-----|

Microbiological characteristics of the test samples of Balyk Marochny at a fixed temperature

| Marochny at a fixed temperature |                |                       |                |   |   |                         |  |  |
|---------------------------------|----------------|-----------------------|----------------|---|---|-------------------------|--|--|
| No.                             | Sample         | MAOAnM,<br>CFU in 1 g | ECBG<br>in 1 g | Sulfite<br>reducing<br>Clostridia<br>in 1 g | Pathogenic<br>microor-<br>ganisms<br>including<br>Salmonella<br>in 25 g | St.<br>aureus<br>in 1 g |  |  |
|                                 |                | On                    | day 1 of       | storage                                     | . 0   |                         |  |  |
| 1                               | Control        | 1.5·10 <sup>2</sup>   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 2                               | Sample<br>1.1  | 1.3·10 <sup>2</sup>   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 3                               | Sample 1. 2    | 8.0·10 <sup>1</sup>   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 4                               | Sample<br>1.3  | <10                   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 5                               | Sample<br>1.4  | <10                   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
|                                 | 1.4            | On                    | day 14 o       | f storage                                   | Iouna   | Iouna                   |  |  |
| 6                               | Control        | 2.9·10 <sup>2</sup>   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 7                               | Sample<br>1.1  | $2.4 \cdot 10^2$      | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 8                               | Sample 1.2     | $1.9 \cdot 10^2$      | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 9                               | Sample<br>1.3  | $4.5 \cdot 10^{1}$    | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 10                              | Sample<br>1.4  | $3.0 \cdot 10^{1}$    | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
|                                 |                | On                    | day 21 o       | f storage                                   |   |                         |  |  |
| 11                              | Control        | $7.2 \cdot 10^2$      | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 12                              | Sample<br>1.1  | $6.4 \cdot 10^2$      | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 13                              | Sample<br>1.2  | $2.5 \cdot 10^2$      | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 14                              | Sample<br>1.3  | $8.5 \cdot 10^{1}$    | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 15                              | Sample<br>1.4  | $6.0 \cdot 10^{1}$    | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
|                                 |                | On                    | day 28 o       | f storage                                   |   |                         |  |  |
| 16                              | Control        | $4.5 \cdot 10^{3}$    | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 17                              | Sample<br>1.1  | 3.9·10 <sup>3</sup>   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 18                              | Sample<br>1.2  | 1.6·10 <sup>3</sup>   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 19                              | Sample<br>1. 3 | $4.4 \cdot 10^2$      | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 20                              | Sample<br>1.4  | $3.5 \cdot 10^{2}$    | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| On day 35 of storage            |                |                       |                |   |   |                         |  |  |
| 21                              | Control        | 1.3·10 <sup>5</sup>   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 22                              | Sample<br>1.1  | 1.1·10 <sup>5</sup>   | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 23                              | Sample<br>1.2  | $5.0.10^{4}$          | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 24                              | Sample<br>1. 3 | $6.8 \cdot 10^2$      | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| 25                              | Sample<br>1.4  | $6.3 \cdot 10^2$      | not<br>found   | not found                                   | not<br>found  | not<br>found            |  |  |
| -                               |                |                       |                |   |   |                         |  |  |

Table 2

Table 3

The results of pH in the test samples of Balyk Marochny at a fixed temperature

| No. | Storage<br>duration,<br>day | Control | Sample<br>1. 1 | Sample<br>1. 2 | Sample<br>1. 3 | Sample<br>1.4 |
|-----|-----------------------------|---------|----------------|----------------|----------------|---------------|
| 1   | 1                           | 5.98    | 5.97           | 5.98           | 5.98           | 5.97          |
| 2   | 14                          | 6.20    | 6.10           | 6.08           | 6,04           | 6.03          |
| 3   | 21                          | 6.25    | 6.22           | 6.18           | 6.15           | 6.10          |
| 4   | 28                          | 6.28    | 6.26           | 6.20           | 6.18           | 6.14          |
| 5   | 35                          | 6.42    | 6.40           | 6.34           | 6.21           | 6.19          |

Organoleptic assessment of the samples was performed in a 5-point assessment scale. The results are presented in Table 3.

The results of organoleptic assessment of samples of Balyk Marochny at a fixed temperature Character-Con-Sample Sample Sample Sample trol istic 1.1 1.2 1.3 1.4 On day 1, 14 and 21 of storage Appearance 5.0 5.0 5.05.03.0 Consistency 5.0 5.0 5.0 5.0 5.0 Cut 5.05.05.0 5.05.0appearance 5.05.0 5.05.0 5.0 Smell Taste 5.0 5.0 5.0 5.0 5.0 Overall 5.05.0 5.0 5.04.6assessment On day 28 of storage 2.0 5.0 3.0 Appearance 3.0 4.0Consistency 4.04.04.05.05.0Cut 4.0 4.0 4.0 5.0 5.0 appearance 5.0 Smell 1.0 1.010 50Taste 1.0 1.01.0 505.0 Overall 2.42.62.85.0 4.6assessment On day 35 of storage Appearance 1.0 1.0 1.0 5.03.0 Consistency 1.0 1.01.0 5.0 5.0Cut 5.0 1.0 1.0 1.0 5.0 appearance Smell 1.0 1.0 1.0 5.0 5.0 Taste 1.0 1.0 1.0 5.0 5.0 Overall 1.0 1.0 1.0 5.0 4.6

Organoleptic studies have shown that post-pasteurization for 1, 2 and 3 minutes does not affect product appearance and attractiveness as well as its taste and aroma. The test samples did not differ from the control sample.

After post-pasteurization for 4 min., the sample 1. 4 showed signs of fat melting that affects the product appearance and attractiveness.

During storage, first signs of spoilage were observed in the control and test samples 1.1 and 1.2 on day 28 of storage. The control sample had a pronounced odor and there were clear signs of spoilage. Samples 1. 1 and 1. 2 had a slight odor.

On day 35 of storage, sample 1.3 had excellent organoleptic characteristics without any sign of spoilage and change of appearance after post-pasteurization. Sample 1.4 was edible, had a good taste and aroma but appearance was worse than that of sample 1.3.

Analyzing all data presented, a conclusion was drawn that optimal duration of post-pasteurization is 3 min.

At the next stage, studies were conducted for a fixed thermal treatment time of 3 min to determine optimal postpasteurization temperature at different temperatures.

The results of microbiological studies of control and test samples within 42 days of storage are presented in Table 4.

Table 4

Microbiological parameters of the test specimens of Balyk Marochny at a fixed duration of thermal treatment

| No.MAO-<br>AnM,<br>1 gMAO-<br>CFU in<br>1 gSulfite<br>BCBG in<br>1 gSulfite<br>reducing<br>in 1 gPathogenic<br>microorgan<br>subuding<br>Subunella in<br>25 gSt. aureus<br>in 1 g1Control2.3·102not foundnot foundnot foundnot foundnot found2Sample 2.11.8·102not foundnot foundnot foundnot foundnot found3Sample 2.21.7·102not foundnot foundnot foundnot found4Sample 2.31.4·102not foundnot foundnot foundnot found5Sample 2.4<10not foundnot foundnot foundnot found6Control4.2·102not foundnot foundnot foundnot found7Sample 2.31.8·102not foundnot foundnot foundnot found8Sample 2.31.8·102not foundnot foundnot foundnot found9Sample 2.31.8·102not foundnot foundnot foundnot found10Sample 2.31.8·102not foundnot foundnot foundnot found11Control8.9·102not foundnot foundnot foundnot found12Sample 2.38.5·102not foundnot foundnot foundnot found13Sample 2.48.5·102not foundnot foundnot foundnot found14Control5.6·102not foundnot foundnot foundnot fo   |                      |            |                    |           |                       |  |           |  |  |
|---|----------------------|------------|--------------------|-----------|-----------------------|--|-----------|--|--|
| 1Control2.3 $\cdot 10^2$ not foundnot foundnot foundnot found2Sample 2.1 $1.8 \cdot 10^2$ not foundnot foundnot foundnot foundnot found3Sample 2.2 $1.7 \cdot 10^2$ not foundnot foundnot foundnot foundnot found4Sample 2.3 $1.4 \cdot 10^2$ not foundnot foundnot foundnot foundnot found5Sample 2.4<10   | No.                  | Sample     | AnM,<br>CFU in     |           | reducing<br>Clostrids | microorgan-<br>isms including<br>Salmonella in |           |  |  |
| 1Control2.3 $\cdot 10^2$ not foundnot foundnot foundnot found2Sample 2.1 $1.8 \cdot 10^2$ not foundnot foundnot foundnot foundnot found3Sample 2.2 $1.7 \cdot 10^2$ not foundnot foundnot foundnot foundnot found4Sample 2.3 $1.4 \cdot 10^2$ not foundnot foundnot foundnot foundnot found5Sample 2.4<10   |                      |            |                    |           |                       |  |           |  |  |
| 3Sample 2.2 $1.7 \cdot 10^2$ not foundnot foundnot foundnot found4Sample 2.3 $1.4 \cdot 10^2$ not foundnot foundnot foundnot foundnot found5Sample 2.4<10   | 1                    | Control    | $2.3 \cdot 10^2$   |           | _                     | not found                                      | not found |  |  |
| 4Sample 2.3 $1.4 \cdot 10^2$ not foundnot foundnot foundnot found5Sample 2.4 $<10$ not foundnot foundnot foundnot found6Control $4.2 \cdot 10^2$ not foundnot foundnot foundnot found7Sample 2.1 $3.6 \cdot 10^2$ not foundnot foundnot foundnot found8Sample 2.2 $2.9 \cdot 10^2$ not foundnot foundnot foundnot found9Sample 2.3 $1.8 \cdot 10^2$ not foundnot foundnot foundnot found10Sample 2.4 $3.5 \cdot 10^1$ not foundnot foundnot foundnot found11Control $8.9 \cdot 10^2$ not foundnot foundnot foundnot found12Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found13Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot found<  | 2                    | Sample 2.1 | $1.8 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 5Sample 2.4<10not foundnot foundnot foundnot foundnot found0Control $4.2 \cdot 10^2$ not foundnot foundnot foundnot foundnot found7Sample 2.1 $3.6 \cdot 10^2$ not foundnot foundnot foundnot foundnot found8Sample 2.2 $2.9 \cdot 10^2$ not foundnot foundnot foundnot foundnot found9Sample 2.3 $1.8 \cdot 10^2$ not foundnot foundnot foundnot found10Sample 2.4 $3.5 \cdot 10^1$ not foundnot foundnot foundnot found11Control $8.9 \cdot 10^2$ not foundnot foundnot foundnot found12Sample 2.1 $8.5 \cdot 10^2$ not foundnot foundnot foundnot found13Sample 2.2 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot found18Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot found19Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot found19Sample 2.4 $2.5 \cdot 10^2$ not   | 3                    | Sample 2.2 | $1.7 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| On day 14 of storage6Control $4.2 \cdot 10^2$ not foundnot foundnot foundnot found7Sample 2.1 $3.6 \cdot 10^2$ not foundnot foundnot foundnot found8Sample 2.2 $2.9 \cdot 10^2$ not foundnot foundnot foundnot found9Sample 2.3 $1.8 \cdot 10^2$ not foundnot foundnot foundnot found10Sample 2.4 $3.5 \cdot 10^1$ not foundnot foundnot foundnot found11Control $8.9 \cdot 10^2$ not foundnot foundnot foundnot found12Sample 2.1 $8.5 \cdot 10^2$ not foundnot foundnot foundnot found13Sample 2.2 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20S  | 4                    | Sample 2.3 | $1.4 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 6Control $4.2:10^2$ not foundnot foundnot foundnot found7Sample 2.1 $3.6:10^2$ not foundnot foundnot foundnot found8Sample 2.2 $2.9:10^2$ not foundnot foundnot foundnot found9Sample 2.3 $1.8:10^2$ not foundnot foundnot foundnot found10Sample 2.4 $3.5:10^1$ not foundnot foundnot foundnot found11Control $8.9:10^2$ not foundnot foundnot foundnot found12Sample 2.1 $8.5:10^2$ not foundnot foundnot foundnot found13Sample 2.2 $8.7:10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6:10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5:10^1$ not foundnot foundnot foundnot found16Control $5.6:10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4:10^3$ not foundnot foundnot found18Sample 2.2 $2.1:10^3$ not foundnot foundnot found19Sample 2.3 $6.4:10^2$ not foundnot foundnot found10Sample 2.4 $2.5:10^2$ not foundnot foundnot found19Sample 2.4 $2.5:10^2$ not foundnot foundnot found19Sample 2.4 $2.5:10^2$ not   | 5                    | Sample 2.4 | <10                | not found | not found             | not found                                      | not found |  |  |
| 7Sample 2.1 $3.6 \cdot 10^2$ not foundnot foundnot foundnot found8Sample 2.2 $2.9 \cdot 10^2$ not foundnot foundnot foundnot found9Sample 2.3 $1.8 \cdot 10^2$ not foundnot foundnot foundnot found10Sample 2.4 $3.5 \cdot 10^1$ not foundnot foundnot foundnot found11Control $8.9 \cdot 10^2$ not foundnot foundnot foundnot found12Sample 2.1 $8.5 \cdot 10^2$ not foundnot foundnot foundnot found13Sample 2.2 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not found<  |                      |            |                    | On day 14 | of storage            |  |           |  |  |
| 8Sample 2.22.9·102not foundnot foundnot foundnot found9Sample 2.3 $1.8\cdot10^2$ not foundnot foundnot foundnot found10Sample 2.4 $3.5\cdot10^1$ not foundnot foundnot foundnot found11Control $8.9\cdot10^2$ not foundnot foundnot foundnot found12Sample 2.1 $8.5\cdot10^2$ not foundnot foundnot foundnot found13Sample 2.2 $8.7\cdot10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6\cdot10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5\cdot10^1$ not foundnot foundnot foundnot found16Control $5.6\cdot10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4\cdot10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1\cdot10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4\cdot10^2$ not foundnot foundnot foundnot found19Sample 2.4 $2.5\cdot10^2$ not foundnot foundnot foundnot found19Sample 2.1 $8.6\cdot10^3$ not foundnot foundnot foundnot found20Sample 2.1 $8.6\cdot10^3$ not foundnot foundnot foundnot found21Control $2.7\cdot10^5$ not foundnot foundnot foun  | 6                    | Control    | $4.2 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 9Sample 2.3 $1.8 \cdot 10^2$ not foundnot foundnot foundnot found10Sample 2.4 $3.5 \cdot 10^1$ not foundnot foundnot foundnot foundnot found11Control $8.9 \cdot 10^2$ not foundnot foundnot foundnot foundnot found12Sample 2.1 $8.5 \cdot 10^2$ not foundnot foundnot foundnot found13Sample 2.2 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found18Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found19Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $7.10^3$ not foundnot foundnot foundnot found19Sample 2.4 $7.10^3$ not foundnot foundnot foundnot found20Sample 2.4 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found21Control  | 7                    | Sample 2.1 | $3.6 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 10Sample 2.4 $3.5 \cdot 10^1$ not foundnot foundnot foundnot found11Control $8.9 \cdot 10^2$ not foundnot foundnot foundnot foundnot found12Sample 2.1 $8.5 \cdot 10^2$ not foundnot foundnot foundnot foundnot found13Sample 2.2 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found19Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.3<  | 8                    | Sample 2.2 |                    | not found | not found             | not found                                      |           |  |  |
| On day 21 of storage11Control $8.9 \cdot 10^2$ not foundnot foundnot foundnot found12Sample 2.1 $8.5 \cdot 10^2$ not foundnot foundnot foundnot found13Sample 2.2 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24<  | 9                    | Sample 2.3 | $1.8 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 11Control $8.9 \cdot 10^2$ not foundnot foundnot foundnot found12Sample 2.1 $8.5 \cdot 10^2$ not foundnot foundnot foundnot found13Sample 2.2 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not fou  | 10                   | Sample 2.4 | $3.5 \cdot 10^{1}$ | not found | not found             | not found                                      | not found |  |  |
| 12Sample 2.1 $8.5 \cdot 10^2$ not foundnot foundnot foundnot found13Sample 2.2 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not   |                      |            |                    |           |                       |  |           |  |  |
| 13Sample 2.2 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8 \cdot $   | 11                   | Control    |                    | not found | not found             | not found                                      | not found |  |  |
| 14Sample 2.3 $5.6 \cdot 10^2$ not foundnot foundnot foundnot found15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not  | 12                   | Sample 2.1 | $8.5 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 15Sample 2.4 $6.5 \cdot 10^1$ not foundnot foundnot foundnot found16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8 \cdot 10^4$ not  | 13                   | Sample 2.2 | $8.7 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| On day 28 of storage16Control $5.6\cdot10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4\cdot10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1\cdot10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4\cdot10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5\cdot10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5\cdot10^2$ not foundnot foundnot foundnot found21Control $2.7\cdot10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6\cdot10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8\cdot10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1\cdot10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7\cdot10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot foundnot found27Sample 2.1 $6.3\cdot10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8\cdot10^4$ not foundnot foundnot foundnot found29Sample 2.3 $4.5\cdot10^4$ not foundnot foundnot foundnot found <td>14</td> <td>Sample 2.3</td> <td><math>5.6 \cdot 10^2</math></td> <td>not found</td> <td>not found</td> <td>not found</td> <td>not found</td> | 14                   | Sample 2.3 | $5.6 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 16Control $5.6 \cdot 10^4$ not foundnot foundnot foundnot found17Sample 2.1 $2.4 \cdot 10^3$ not foundnot foundnot foundnot found18Sample 2.2 $2.1 \cdot 10^3$ not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot found28Sample 2.2 $4.8 \cdot 10^4$ not foundnot foundnot foundnot found29Sample 2.3 $4.5 \cdot $  | 15                   | Sample 2.4 | $6.5 \cdot 10^{1}$ | not found | not found             | not found                                      | not found |  |  |
| $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$  |                      |            |                    | On day 28 | of storage            |  |           |  |  |
| 18Sample 2.22.1 \cdot 10^3not foundnot foundnot foundnot found19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8 \cdot 10^4$ not foundnot foundnot foundnot found29Sample 2.3 $4.5 \cdot 10^4$ not foundnot foundnot foundnot found   | 16                   | Control    | $5.6 \cdot 10^4$   | not found | not found             | not found                                      | not found |  |  |
| 19Sample 2.3 $6.4 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot foundOn day 35 of storage21Control $2.7 \cdot 10^5$ not foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8 \cdot 10^4$ not foundnot foundnot foundnot found29Sample 2.3 $4.5 \cdot 10^4$ not foundnot foundnot foundnot found   | 17                   | Sample 2.1 | $2.4 \cdot 10^{3}$ | not found | not found             | not found                                      | not found |  |  |
| 20Sample 2.4 $2.5 \cdot 10^2$ not foundnot foundnot foundnot found21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8 \cdot 10^4$ not foundnot foundnot foundnot found29Sample 2.3 $4.5 \cdot 10^4$ not foundnot foundnot foundnot found  | 18                   | Sample 2.2 | $2.1 \cdot 10^{3}$ | not found | not found             | not found                                      | not found |  |  |
| On day 35 of storage21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8 \cdot 10^4$ not foundnot foundnot foundnot found29Sample 2.3 $4.5 \cdot 10^4$ not foundnot foundnot foundnot found  | 19                   | Sample 2.3 | $6.4 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 21Control $2.7 \cdot 10^5$ not foundnot foundnot foundnot found22Sample 2.1 $8.6 \cdot 10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8 \cdot 10^4$ not foundnot foundnot foundnot found29Sample 2.3 $4.5 \cdot 10^4$ not foundnot foundnot foundnot found  | 20                   | Sample 2.4 | $2.5 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 22Sample 2.1 $8.6\cdot10^3$ not foundnot foundnot foundnot found23Sample 2.2 $7.8\cdot10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1\cdot10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7\cdot10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.1 $6.3\cdot10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8\cdot10^4$ not foundnot foundnot foundnot found29Sample 2.3 $4.5\cdot10^4$ not foundnot foundnot foundnot found   |                      |            |                    | On day 35 | of storage            |  |           |  |  |
| 23Sample 2.2 $7.8 \cdot 10^3$ not foundnot foundnot foundnot found24Sample 2.3 $7.1 \cdot 10^3$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found25Sample 2.4 $8.7 \cdot 10^2$ not foundnot foundnot foundnot found26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.1 $6.3 \cdot 10^4$ not foundnot foundnot foundnot found28Sample 2.2 $4.8 \cdot 10^4$ not foundnot foundnot foundnot found29Sample 2.3 $4.5 \cdot 10^4$ not foundnot foundnot foundnot found   | 21                   | Control    | $2.7 \cdot 10^{5}$ | not found | not found             | not found                                      | not found |  |  |
| $\begin{array}{c ccccccccccccccccccccccccccccccccccc$   | 22                   | Sample 2.1 | $8.6 \cdot 10^3$   | not found | not found             | not found                                      | not found |  |  |
| 25Sample 2.48.7 · 10²not foundnot foundnot foundnot foundOn day 42 of storage26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.16.3 · 10 <sup>4</sup> not foundnot foundnot foundnot found28Sample 2.24.8 · 10 <sup>4</sup> not foundnot foundnot foundnot found29Sample 2.34.5 · 10 <sup>4</sup> not foundnot foundnot foundnot found  | 23                   | Sample 2.2 | $7.8 \cdot 10^3$   | not found | not found             | not found                                      | not found |  |  |
| On day 42 of storage   26 Control full growth not found not found not found not found   27 Sample 2.1 6.3·10 <sup>4</sup> not found not found not found not found   28 Sample 2.2 4.8·10 <sup>4</sup> not found not found not found not found   29 Sample 2.3 4.5·10 <sup>4</sup> not found not found not found not found   | 24                   | Sample 2.3 | $7.1 \cdot 10^{3}$ | not found | not found             | not found                                      | not found |  |  |
| 26Controlfull<br>growthnot foundnot foundnot foundnot found27Sample 2.16.3·10 <sup>4</sup> not foundnot foundnot foundnot found28Sample 2.24.8·10 <sup>4</sup> not foundnot foundnot foundnot found29Sample 2.34.5·10 <sup>4</sup> not foundnot foundnot foundnot found   | 25                   | Sample 2.4 | $8.7 \cdot 10^2$   | not found | not found             | not found                                      | not found |  |  |
| 26Controlgrowthnot foundnot foundnot foundnot found27Sample 2.16.3·10 <sup>4</sup> not foundnot foundnot foundnot found28Sample 2.24.8·10 <sup>4</sup> not foundnot foundnot foundnot found29Sample 2.34.5·10 <sup>4</sup> not foundnot foundnot foundnot found   | On day 42 of storage |            |                    |           |                       |  |           |  |  |
| 28Sample 2.24.8 · 10 <sup>4</sup> not foundnot foundnot foundnot found29Sample 2.34.5 · 10 <sup>4</sup> not foundnot foundnot foundnot found  | 26                   |            |                    | not found | not found             | not found                                      | not found |  |  |
| $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$  | 27                   | Sample 2.1 | $6.3 \cdot 10^4$   | not found | not found             | not found                                      | not found |  |  |
|   | 28                   | Sample 2.2 | $4.8 \cdot 10^{4}$ | not found | not found             | not found                                      | not found |  |  |
| 20 Sample 2.4 2.4 10 <sup>3</sup> pot found not found not found not found   | 29                   | Sample 2.3 |                    | not found | not found             | not found                                      |           |  |  |
| 30 Sample 2.4 2.4·10° not iound not iound not iound not iound   | 30                   | Sample 2.4 | $2.4 \cdot 10^3$   | not found | not found             | not found                                      | not found |  |  |

assessment

#### Table 6

The results of organoleptic evaluations of the samples of Balyk Marochny at a fixed duration of thermal treatment

| Characteristic       | Control | Sample 2.1     | Sample 2. 2 | Sample 2.3 | Sample 2.4 |  |
|----------------------|---------|----------------|-------------|------------|------------|--|
| On day 28 of storage |         |                |             |            |            |  |
| Appearance           | 3.0     | 3.0            | 3.0         | 5.0        | 5.0        |  |
| Consistency          | 4.0     | 4.0            | 4.0         | 5.0        | 5.0        |  |
| Cut appearance       | 3.0     | 4.0            | 4.0         | 5.0        | 5.0        |  |
| Smell                | 1.0     | 1.0            | 1.0         | 5.0        | 5.0        |  |
| Taste                | 1.0     | 1.0            | 1.0         | 5.0        | 5.0        |  |
| Overall assessment   | 2.4     | 2.6            | 2.6         | 5.0        | 5.0        |  |
|                      |         | On day 35 of s | storage     |            |            |  |
| Appearance           | 1.0     | 1.0            | 1.0         | 2.0        | 5.0        |  |
| Consistency          | 1.0     | 1.0            | 1.0         | 3.0        | 5.0        |  |
| Cut appearance       | 1.0     | 1.0            | 1.0         | 3.0        | 5.0        |  |
| Smell                | 1.0     | 1.0            | 1.0         | 1.0        | 4.0        |  |
| Taste                | 1.0     | 1.0            | 1.0         | 1.0        | 5.0        |  |
| Overall assessment   | 1.0     | 1.0            | 1.0         | 2,0        | 4.8        |  |
|                      |         | On day 42 of s | storage     |            |            |  |
| Appearance           | 1.0     | 1.0            | 1.0         | 1.0        | 3.0        |  |
| Consistency          | 1.0     | 1.0            | 1.0         | 1.0        | 2.0        |  |
| Cut appearance       | 1.0     | 1.0            | 1.0         | 1.0        | 2.0        |  |
| Smell                | 1.0     | 1.0            | 1.0         | 1.0        | 1.0        |  |
| Taste                | 1.0     | 1.0            | 1.0         | 1.0        | 1.0        |  |
| Overall assessment   | 1.0     | 1.0            | 1.0         | 1.0        | 1.8        |  |

The presented microbiological studies confirm the earlier conclusion that the samples subjected to post-pasteurization have a lower degree of microbial contamination than that of the control specimen.

Parameters of thermal treatment of samples 2.1 and 2.2 were found ineffective enough to significantly reduce number of mesophilic aerobic and conditionally anaerobic microorganisms. Shelf life of these samples was the same as that of the control sample which exceeded the allowable level of  $10^3$  CFU/g on day 28.

Comparison of sample 2.3 with the control sample has shown that the test sample had better microbiological characteristics and its shelf life was 7 days longer than that of the control one.

The sample 2.4 had the best results of post-pasteurization. It was edible on day 35 of storage and did not exceed the maximum permissible rate of MAOAnM. However, the norm was exceeded on day 42 of storage,

The results of studies of moisture content in samples at all stages of storage are presented in Table 5.

#### Table 5

Characteristics of mass fraction of moisture in samples of Balyk Marochny at a fixed duration of thermal treatment, %

| No. | Storage<br>duration,<br>day | Control | Sample<br>2. 1 | Sample<br>2. 2 | Sample<br>2. 3 | Sample<br>2.4 |
|-----|-----------------------------|---------|----------------|----------------|----------------|---------------|
| 1   | 1                           | 81.3    | 81.4           | 80.1           | 78.1           | 81.2          |
| 2   | 14                          | 81.6    | 80.3           | 79.8           | 81.3           | 79.1          |
| 3   | 21                          | 79.7    | 79.0           | 80.5           | 79.5           | 78.7          |
| 4   | 28                          | 80.3    | 81.6           | 80.2           | 79.2           | 80.9          |
| 5   | 35                          | 80.8    | 79.6           | 81.4           | 79.6           | 79.0          |
| 6   | 42                          | 79.4    | 80.3           | 80.9           | 79.9           | 80.3          |

The results of the study of mass fraction of moisture show that post-pasteurization does not actually affect amount of free moisture in the product.

Control and test samples from day 1 to day 21 of storage had excellent organoleptic characteristics and a total score of 5 points. The results of organoleptic studies at further storage duration are presented in Table 6.

The results given in Table 6 indicate that the control sample had pronounced signs of spoilage on day 28 as at the previous stage of the study. Samples 2. 1, 2. 2 were also inedible during this storage period and there was an unpleasant odor of the product.

Organoleptic studies on day 35 of storage indicated that only sample 2.4 had excellent characteristics, it was fresh and edible, and the control sample had very pronounced signs of spoilage.

On day 42 of storage, sample 2.4 had signs of spoilage, namely, a slight unpleasant odor, so this sample was inedible. 6. Discussion of results obtained in the study of influence of post-pasteurization conditions on shelf life of gourmet meat products

Choice of combinations of processing temperature and time for each sample was made on the basis of published data and the studies performed in [32]. The shelf life of gourmet meat products was determined on the basis of comprehensive study methods characterizing microbiological state, organoleptic characteristics and physicochemical parameters, namely concentration of hydrogen ions and mass fraction of moisture.

According to microbiological studies, pathogenic and conditionally pathogenic microorganisms given in Tables 1, 2 were not found after post-pasteurization in the test specimens throughout the shelf life. Thermal treatment in the temperature range of 75–90 °C during 1–4 min. as well as during 3 min. at 75–85 °C is of low efficiency. This fact is evidenced by the data obtained in studies of the MAOAnM characteristic and given in Tables 1, 4.

Reduction of microbial contamination occurs but not enough to extend shelf life of a gourmet meat product. This fact is associated with temperature of the product surface insufficient to inactivate the microbiota [33].

From a microbiological point of view, it is the most effective to use treatment at 90 °C during 4 min., however, changes in appearance of the product must be taken into account. It was established that heating the product surface for more than 3 min. leads to fat melting [11, 34].

Proceeding from analysis of the results obtained in microbiological and organoleptic studies, it can be stated that 90 °C and 3 min are optimal parameters of post-pasteurization treatment. For the storage period from 1 to 35 days, the test specimens subjected to such conditions of thermal treatment did not exceed the MAOAnM normative characteristic, had excellent organoleptic characteristics and were edible.

Studies of mass fraction of moisture in the samples subjected and not subjected to post-pasteurization were carried out with consideration that release of moisture from the product may be possible depending on thermal treatment. Release of moisture depends on the moisture-binding capacity of meat protein after its denaturation. This characteristic is sensitive to thermal effects, i. e. the combination of time and heating rate [20, 35]. The results given in Table 5 show that contents of moisture in the test and control samples differed insignificantly.

During the entire period of storage of the control and test samples, studies were conducted to determine concentration of hydrogen ions. The results of pH studies indicate that post-pasteurization does not affect active medium reaction. During storage, there is a slightly more rapid pH growth in the control samples compared to test ones. On day 35 of storage, pH in the control sample reached a value of 6.42. At the same time, the test sample treated at 90 °C for 3 min. had pH of 6.21.

Dynamics of pH shift to the alkaline side may be caused by development of putrefactive microbiota. Under the action of microorganism enzymes, chemical transformations occur, e. g. accumulation of organic acids that affect pH [20].

Thus, it was established that heating temperature of 90 °C and time of 3 min form rational conditions of thermal treatment of ready-to-eat vacuum-packed gourmet meat products. These parameters of thermal treatment make it possible to extend shelf life of whole-muscle gourmet meat products from 25 to 35 days with no changes in organoleptic

characteristics and moisture syneresis into vacuum of the product packaging.

According to the study results, a patent on useful model No. 125878 The Method for Production of Whole-muscle Pork Products was obtained [36].

The study results show that thermal treatment of readyto-eat gourmet meat products which does not cause changes in the product appearance can be applied just for a short time. The issue of post-pasteurization of high-fat gourmet meat products requires studies.

Further studies may be aimed at determination of quality and patterns of spoilage of the fat component of the product as well as possible structural changes in the meat tissue.

# 7. Conclusions

1. Rational temperature and time factors of influence of post-pasteurization on development of microbiota immediately after treatment and during storage were determined: treatment temperature of 90 °C and duration of 3 min.

2. Based on microbiological, physicochemical, and organoleptic parameters, it was shown that the developed post-pasteurization regimes improve the product quality and safety. Rational post-pasteurization conditions do not alter organoleptic characteristics such as juiciness, color, taste, appearance and do not cause melting of fat under packaging.

3. The possibility of extending shelf life of whole-muscle gournet meat products was proved and a rational shelf life from 25 to 35 days without a change of its quality and safety was established.

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