USE OF BACTERIOPHAGES FOR FOOD PROTECTION FROM MICROBIOLOGICAL SPOILAGE

Elhedmi Abdulkhaleg [1] **Samira Guma Jrad**[2]

Abstract: The article has been studied the ability of bacteriophages, specific to bacteria of the genus *Pseudomonas* to prevent microbiological spoilage of protein-containing food products not subjected to heat treatment (beef, poultry and fish). It is shown that bacteria protects these products from microbiological damage under the influence of host bacteria.

Key words: foods, microbiological spoilage, bacterial, *Pseudomonas*, bacteriophages.

Introduction:

Pseudomonas strains of bacteria have shown to play a key role in milk spoilage, slaughtered poultry and meat, eggs and fish. This study aims at detecting the presence of *Pseudomonas* bacteria in various objects. Microbiological studies have shown that these bacteria (*P. fluorescens, P. aeruginosa*) play a key role in milk spoilage (Ferreira,2013), (V. N. Leontiev et al., 2013), slaughtered poultry and meat, eggs and fish (M. Anbalagan 2014),

[1] - Staff member at Higher Institute of Medical Professions. Al-Qarah Poli, Libya.

[2] - Staff member at faculty of Physical Education and Sports Sciences, Tripoli University, Libya

(A. E. Elhedmi,2015). A crucial task is to expand the collection of bacteriophages capable to lyze food spoilage bacteria and to study their biological characteristics.

Looking for environmentally friendly ways to decontaminate food products using different phages will increase the shelf life and control spoilage.

Microbial spoilage of protein-contained food is one of the most serious reasons of food loss in the world. Bacteria *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* are the most common causative agents of this process (M. M. Medzhidov, 2003). Temperature decrease to 4°C in the chambers of refrigerators is used most often to prolong shelf-life of food products. However, these measures do not greatly inhibit the growth of the above bacteria. Therefore, enhancing food spoilage protection tools seems to be an urgent task. Bacteriophage-based preparations could become some of these tools, because they are not toxic to humans and highly specific for bacteria (Ferreira,2013).

Given the significant prevalence and circulation in the nature of *Pseudomonas*, much attention is paid to the detection of these microorganisms in various sites.

Microbiological studies have shown that bacteria of the genus *Pseudomonas* (*P. fluorescens, P. aeruginosa*) play a key role in spoilage of milk (G. F. Levanova et al., 1995), (F. Gerkhardt, 1981), (L. Franzetti and M. Scarpellini, 2007), meat of slaughter animals and poultry, eggs, fish (A.B.

Ferreira,2013), (M. Anbalagan, 2014), (A.V. Kostyleva and O.Y. Kuznetsov, 2011).

P. fluorescens and *P. aeruginosa* are psychrophilic, obligate-aerobic microorganisms that can reproduce in products under refrigerated storage. These bacteria secrete active enzymes that break down proteins and lipids. They are antagonists of many bacteria and mold.

Over the past decade, there has been a significant increase in bacteriophages as a preventive and curative drugs. This circumstance is due to the steadily growing resistance of bacteria to antibiotics. In conditions global antibiotic resistance, bacteriophages can be effective means for treatment and prevention of many bacterial infections. For therapy bacterial phages successfully used, including industrially produced.

The use of food contaminated with pseudo-mana, if available predisposing factors can lead to infections (Krasilnikov,2011). Therefore, in the present The development of biopharmaceuticals based on bacteriophages is being storage of fruits, vegetables, meat, fish in the process of their processing before packaging production at the enterprises (phage bioprocessing) (AG. Shestakov,2010) (Kiseleva, 2015) (Gabrilovich, 1973).

Our work objective is to analyze bacteriophages applicability of protect protein-contained food from spoilage caused by bacteria *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*.

The urgent task is to expand the collection of bacteriophages capable of lysing bacteria that cause food spoilage and the study of their biological properties. Search for environmentally friendly ways of decontamination of food products using different phages will increase the shelf life and reduce spoilage.

Previously, we have isolated bacteria *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* (the collection strains) from meat, fish and poultry samples exposed to microbiological spoilage.

Materials and methods

Bacteriophages isolated from spoiled poultry, beef and carp samples were the subjects of the study. 20 g of the weighed sample were inoculated with 20 µl of *Pseudomonas* sp. diluted 24 hrs culture and incubated at 30°C for 2 days. The obtained sample was homogenized in 10 ml physiological solution then the cells and coarse particles were precipitated by centrifugation at 6000 min^{-1} for 15 min. Chloroform (20:1) was added to the lyzate, shaken vigorously for 1 minute, left for 20-60 min at room temperature then centrifuged again to obtain a clarified lyzate.

Phagolysate titre and the form and bacteriophage negative colonies were determined by Gracia agar-layer technique (A.E. Elhedmi et al., 2016).

About 2 g of heat untreated protein-contained food samples: beef, poultry and fish were placed into Petri dishes and undergone the following treatments: inoculated with bacteria *Pseudomonas fluorescens* and *Pseudomonas*

aeruginosa (control), treated with bacteriophage suspensions (BV_{12} – isolated from beef, BV_{25} – isolated from poultry, BV_{55} – isolated from fish); treated with suspensions of bacteriophages with their host cells (BV^{B}_{12} – bacteriophages with *Pseudomonas aeruginosa* bacteria, BV^{B}_{25} – bacteriophages with *Pseudomonas aeruginosa* bacteria and BV^{B}_{55} – bacteriophages with *Pseudomonas fluorescens* bacteria). After that Petri dishes with the samples were stored at 4°C (9 days) and at 30°C (5 days).

Results and discussion:

our results revealed that samples treated with *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* cells exhibited organoleptic deterioration. On the third day, the surface of the samples became mucous and mucus appeared on poultry and fish samples inoculated with *Pseudomonas aeruginosa* bacteria. Color changes began to appear on the samples on the 5th–6th day of storage and faint odor appeared on the 3rd–4th day.

With experienced observed about the same changes in organoleptic properties as in the blank experiment. Suspensions of bacteriophages in 1.3–4 times increased the time of appearance of different signs of microbial contamination of samples at storage temperatures of 4 ° C and 30 ° C.

Thus, the performed study showed that the processing of samples of protein-containing products with suspensions of bacteriophages of bacteria of the genus *Pseudomonas* protects these products from contamination. The

combined use of three bacteriophages increased the shelf life of all samples tested at 4 ° C and poultry and beef samples at 30 ° C.

The samples treated with phage suspension and the samples treated with phage suspension plus host cells demonstrated almost the same results – mucous appeared on the samples surface only on day 6 – 8, simultaneously with changes in color and odor. (Figure.1)

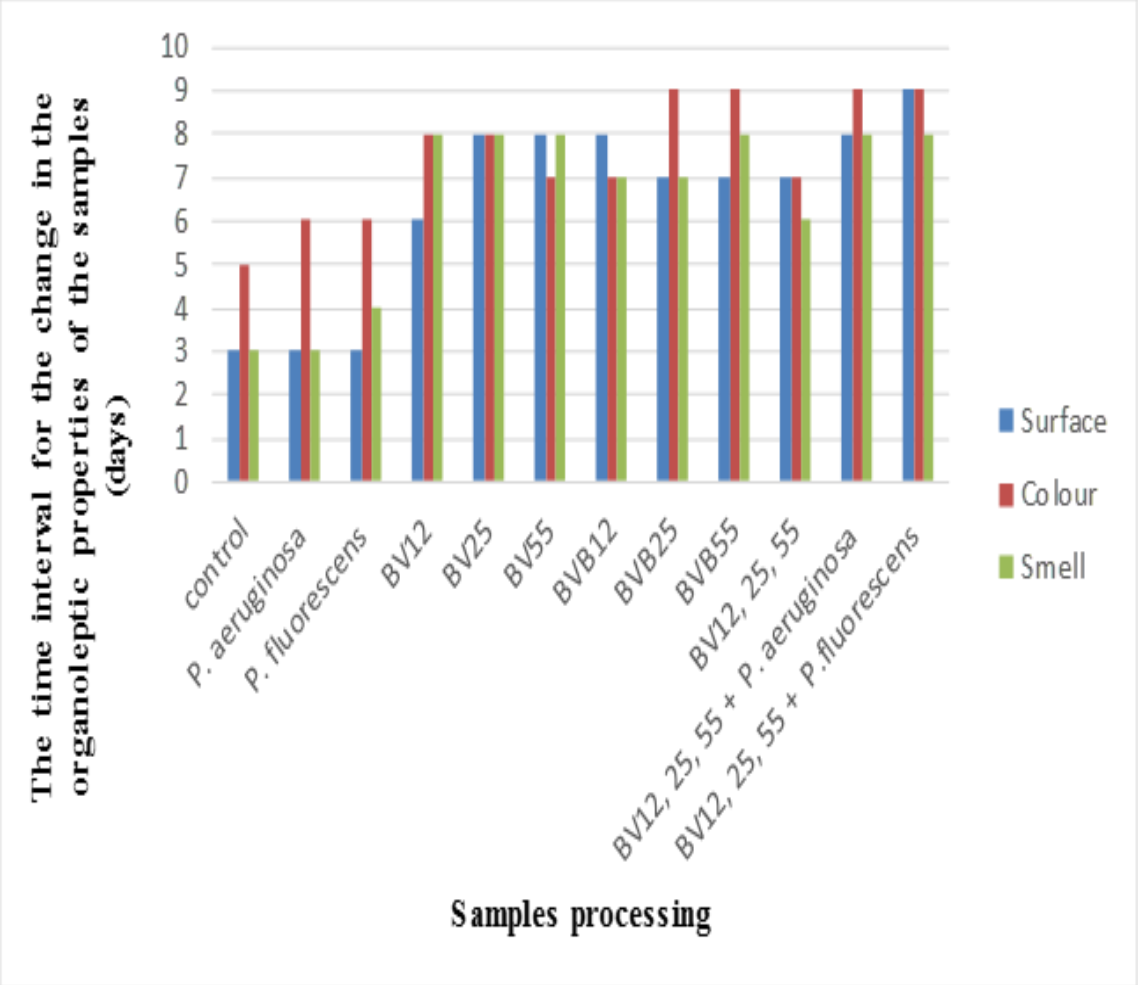

Fig 1 Change in the organoleptic properties of beef samples after treatment with suspensions of bacteria and bacteriophages (at 4 ° C)

Similar results were obtained with similarly treated samples stored at 30°C. But treated with *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* samples organoleptic deterioration were already observed at the end of the first day; when treated with phage suspension and phage suspension plus host cells – on day 2 – 3. (Figure.2)

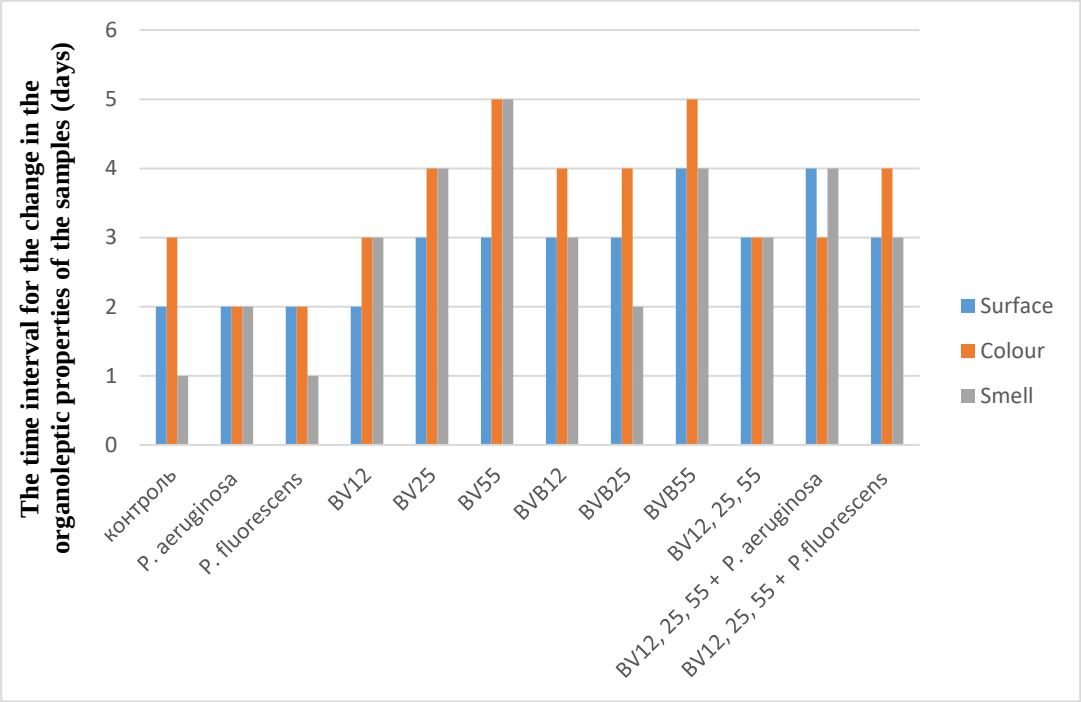

Fig 2 Change in the organoleptic properties of beef samples after treatment with suspensions of bacteria and bacteriophages (at 30 ° C)

Established $1.3 - 4$ fold increase in the shelf life of protein-containing foodstuffs infected by different parameters, *P. fluorescens* or *P. aeruginosa* at 4 ° C and 30 ° C as a result of treatment with suspensions of bacteriophages. This allows the use of bacteriophages BV-$_{12}$, BV-$_{25}$ and BV-$_{55}$ for the development of a biological product that protects food from microbial contamination.

The results obtained can be used to develop a biological product that protects protein-containing foods that have not undergone heat treatment from microbial contamination.

Conclusions:

The developed model system allowed to adequately study the influence of bacteriophages on the process of microbial contamination of protein-containing food products.

A comparative analysis of the effectiveness of protection of samples of protein-containing foods with different bacteriophages and their mixture from microbial contamination caused by infection of *P. aeruginosa* and *P. fluorescens* strains by bacteria has been performed.

The obtained experimental results confirm the possibility of protecting protein-containing foods that have not undergone heat treatment and can be used in the creation of a biopreparation.

For practical use in the biopreparation, strains of bacteriophages BV-$_{12}$, BV-$_{25}$ and BV-$_{55}$, which protect protein-containing products from microbial

contamination, are recommended. The use of such a biopreparation can ensure an increase in shelf life and improve the taste of food products when stored for a long time in a frozen state (phage bioprocessing).

Protein-contained food treatment with suspensions of bacteriophages resulted in twofold increase of food shelf life at 4°C as well as at 30°C.

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