The Impact of the Treatment Method of Root Crops on Micro flora during their Storage

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Abstract. The study aims at determining the effect of the treatment method of root crops before storage, as well as storage parameters, on the dynamics of their microflora, namely, the quantity of bacterial microflora, mesophilic aerobic and optionally anaerobic microorganisms (MAaOAM), as well as molds. When storing garden carrot at t = +(2±1) °C (during 56 days), the amount of bacterial microflora of the samples subjected to integrated treatment decreased by 2.5 times by the end of storage; the number of mold fungi decreased twice compared to the control. When storing garden carrot at t = +(25±1) °C (for 21 days) the amount of bacterial microflora in the samples treated by electromagnetic fields of extremely low frequencies (with the following parameters: frequency – 28 Hz, the treatment time – 5 min, the magnetic induction – 12 mTl), and by Vitaplan biologic preparation (at the concentration of 10^6 CFU/g, and in the amount of 2.5 ml/kg), decreased by 2.1 times, while the number of mold fungi reduced by 1.5 times. When storing garden beet at t = +(2±1) °C for 56 days, the amount of bacterial microflora of samples, subjected to integrated treatment for 5 min at a frequency of 15, 24, and 30 Hz, and magnetic induction of 9 mTl, as well as treatment with Bactofit biological preparation (at the concentration of 10^6 CFU/g in amount of 2.5 ml/kg), decreased by 1.5 times compared to the control, while the number of mold fungi decreased by 2.3 times. When storing garden beet at t = +(25±1) °C (for 21 days), the quantity of bacterial microflora of samples, subjected to integrated treatment, was by 2.8 times lower compared to the control, while the number of mold fungi reduced by 1.8 times. It has been revealed that the integrated treatment of root crops with biopreparations and extremely low frequency (ELF) electric and magnetic fields (EMF) more effectively inhibits the development of pathogenic microflora compared to treatment only with biopreparations or treatment only by ELF-EMF. Significant retardation of growth rates was revealed in both bacterial and fungal pathogenic microflora during storage of root crops at different temperatures.

***Index Terms*: storage, microbiological spoilage, pathogenic microorganisms, treatment, biological control, physical methods, root crops.**

I. INTRODUCTION

The safety of crop products largely depends on the treatment method before storage and is provided by an optimal combination of various factors. The processes during storage are largely influenced by the quantitative and qualitative composition of the microflora. Crop products can be contaminated with pathogenic microorganisms of various types, causing undesirable reactions, impairing taste, smell, color, appearance, and leading to spoilage [1]. The use of a natural or controlled microbiota to extend shelf life and improve food safety is defined as biological conservation or biological control. Research in the field of biological control of post-harvest diseases is based on the detection of microorganisms, which are antagonists of phytopathogens that infect crop products. Spores of pathogenic microorganisms germinate quickly enough and colonize wound surfaces rich in sugars and other nutrients. There is a number of methods to adjust the composition of microflora through the use of microbial antagonists of different species. Known biological control products include Candida oleophila (Aspire, Ecogen, Langhorne, PA, US), Cryptococcus albidus (YieldPlus, Lallemand, Montreal, Canada), Candida sake (Candifruit, IRTA, Lleida, Spain), Pseudomonas syringae Van Hall (BioSave, JET Harvest, Longwood, FL, US) – Aspire, YieldPlus, Candifruit and BioSave [2]-[6]. For biological control of pomiferous fruit during storage, it is proposed to use Aureobasidium pullulans (BoniProtect, Bio-Ferm, Tulln, Austria) [7]. For biological control of fruits and vegetables, Metschnikowia fructicola is used (Shemer, Bayer, Leverkusen, Germany) [3]. For potatoes, Pseudomonas fluorescens and P. viridiflava are used, which inhibit the growth of L. monocytogenes. The authors [4] have revealed the antagonistic activity of Bacillus subtilis IMP 215 strain against Sclerotinia sclerotiorum, Alternaria radicina, and Erwinia carotovora phytopathogens.

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Antimicrobials of microbial origin include Natamycin and Nisin. Physical methods of inactivation of microorganisms due to exposure to electromagnetic fields do not directly affect the product, providing a targeted effect on certain categories of microorganisms while maintaining the basic characteristics of food products. Gayán E. et al. [5] have shown that the combination of ultraviolet light and moderate temperatures (below 60 °C) can lead to a 99.99% (4D) decrease in *Salmonella enterica* bacterial content. Among nonthermal treatment technologies, pulsed electric fields (PEF) are the most studied and promising processes providing inhibition of the development of pathogenic microorganisms. It has been established that, on the one hand, microbial inactivation is affected by the state of microorganisms – type, concentration, stage of development and, on the other hand, physical factors – electric field strength and processing time, environmental factors – electrical conductivity, pH [6].

Known technologies of nonthermal treatment for the rapid inactivation of microorganisms involve the use of intense and short pulses of a wide spectrum [21].

Treatment with ultraviolet light is a nonthermal method using light within the electromagnetic spectrum from 100 to 400 nm. It is a widespread technology for extending the shelf life of food products [22], [23].

In addition, to ensure long-term storage of food products, a promising way is ultraviolet radiation (spectrum from 100 to 400 nm). The resulting photoproducts interrupt DNA transcription [8].

Ionizing radiation (for example, gamma, and electron radiation, as well as X-rays) is a flexible and efficient sterilization technology [9].

Ionizing radiation acts directly and indirectly.

In the first case, cell division is prevented due to damage to DNA by ionizing particles. In the second case, ionizing particles lead to the formation of active hydrogen radicals, hydrated electrons, which leads to cell destruction [10].

The use of ionizing radiation allows one to control many types of spore-forming microorganisms.

The main components of food exposed to ionizing radiation are water, carbohydrates, proteins and lipids. Lowering the treatment temperature prevents undesired chemical changes.

Microwave radiation is widely used in the household microwave ovens. It is believed that when micro-organisms are inactivated, microwaves have both thermal and nonthermal effects [31].

Heat treatment prevented the rotting of apples inoculated by *P. expansum*. The heating also improved biological control with thermally-stable yeast when applied to the apples [34].

The combined use of antagonistic yeasts of the genera Pseudozyma, Aureobasidium and Metschnikowia in combination with HWD has shown to be highly effective in preventing microbiological spoilage of fruits [12].

There are some known studies confirming the effectiveness of the combined treatment using both physical and microbiological factors.

When treating *P. expansum* spores by gamma irradiation, a dose of 0.6 kGy was required to completely inhibit spore germination. Further treatment of *P. expansum* spores by gamma irradiation, and subsequent incubation with *P. fluorescens* suspension at a ratio of 3:5 for 48 hours made it possible to suppress completely spore germination with exposure to a dose of just 0.3 kGy. Integration of irradiation and antagonist can lead to more effective control of pathogens [13].

The aim of the present study is to determine the effect of the treatment method of root crops before storage on the dynamics of microflora, namely, the quantity of bacterial microflora, MAaOAM and molds, as well as to define most optimal storage parameters.

II. MATERIALS AND METHODS

For research, root crops of Abaco carrot and Rhonda beet were selected. These vegetables were grown in the Dinsky District of the Krasnodar Region and were without mechanical damage and signs of infection and physiological diseases.

To study the dynamics of microbial contamination of the vegetables' surface, root crops of carrot and beet, not affected by diseases and not having mechanical damage, were placed in closed transparent plastic containers in an amount of 20 pieces per sample. A vessel with liquid was also placed into the container to ensure humidity.

The reference samples were not subjected to any treatment, while the studied samples were treated either with biological preparations, or with extremely low-frequency electromagnetic fields (ELF-EMF), or were subjected to complex treatment with both a biological preparation and ELF-EMF. Parameters and treatment options are given in Table 1.

Table 1. Characteristic of the methods used to prepare garden carrot for storage, and storage parameters

Quantity of sample	Treatment and storage parameters	
	Carrot roots	**Beet roots**
Reference		
1	t = +(2±1) °C, W = 90%, for 56 days	
2	t = +(25±1) °C, W = 50%, for 21 days	
Treatment with a biological preparation 10^6 CFU/g, in the amount of 2.5 ml/kg, solution temperature 23–25°C		
Vitaplan		Bactofit
3	t = +(2±1) °C, W = 90%, for 56 days	

4	t = +(25±1) °C, W = 50%, for 21 days
Treatment with ELF-EMF	
Frequency of 28 Hz, the treatment time of 30 min, a magnetic induction of 12 mTl	The processing time of 10 min for each of the successively applied frequencies of 15, 24, and 30 Hz, at the magnetic induction of 9 mTl
5	t = +(2±1) °C, W = 90%, for 56 days
6	t = +(25±1) °C, W = 50%, for 21 days
Complex treatment with ELF-EMF and biological preparation	
Frequency of 28 Hz, the treatment time of 5 min, a magnetic induction of 12 mTl + Vitaplan biological preparation, 10^6 CFU/g, in amount of 2.5 ml/kg	The processing time of 5 min for each of the successively applied frequencies of 15, 24, and 30 Hz, at the magnetic induction of 9 mTl + Bactofit preparation, 10^6 CFU/g, in the amount of 2.5 ml/kg
7	t= +(2±1) °C, W = 90%, for 56 days
8	t =+(25±1) °C, W =50%, for 21 days

For the treatment of root crops by ELF-EMF according to previously established parameters of ELF-EMF, a laboratory experimental setup for plant raw materials was used [37]-[38]. After treatment, one part of the root crop samples was stored at a temperature of +(2±1) °C, while another – at t = +(25±1) °C.

During storage the number of microorganisms on the surface of root crops, namely, mesophilic aerobic and optionally anaerobic microorganisms (MAaOAM), as well as molds were periodically determined in CFU/g.

The studies were conducted in accordance with the following state standards of the Russian Federation: 10444.12-2013, 10444.15-94, and 26669-85 [39]-[41].

Determination of microbial contamination of the product sample surface was carried out by rinse sampling from the root crops surface with the peptone-salt solution. The obtained washings were used for preparing a series of tenfold dilution. The samples of (1±0.1) cm^3 were taken from the dilutions and sown in parallel in two Petri dishes. To identify the number of MAaOAM, inoculations were covered by molten nutrient agar medium cooled to (45±0.1) °C, while to identify the molds we used melted glucose agar, cooled to (45±0.1) °C. The inoculations were cultured at (30±1) °C for 72 hours for the cultivation of MAaOAM, and at t = +(24±1) °C for 5 days – for molds. After the specified thermostating time, the grown colonies of microorganisms were counted.

Studies were performed at intervals of every 14 days during 56 days for root crops stored at t = +(2±1) °C, and every 7 days during 21 days for root crops stored at t = +(25±1) °C.

III. RESULTS

The conducted study aims at revealing the influence of the root crops treatment method before storage on their microbiological indicators.

Fig. 1 shows the dynamics of the microbial population of the carrot roots surface during storage at a t= +(2±1)°C, (samples No. 1, 3, 5, 7).

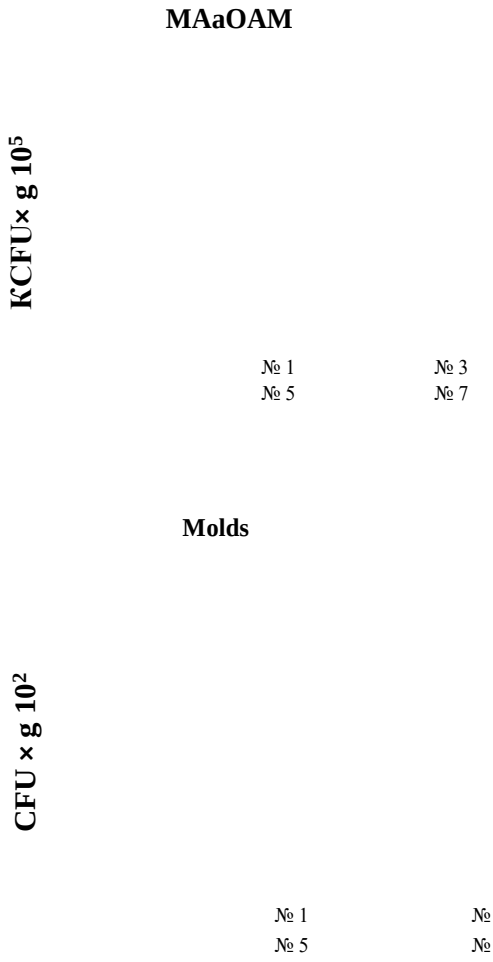


Fig. 1. Dynamics of the microbial population of the carrot roots surface during storage at t = +(2±1) °C (samples No 1, 3, 5, 7)

Fig. 2 shows the dynamics of the microbial population of the carrot roots surface during storage at a t=+(25±1) °C.

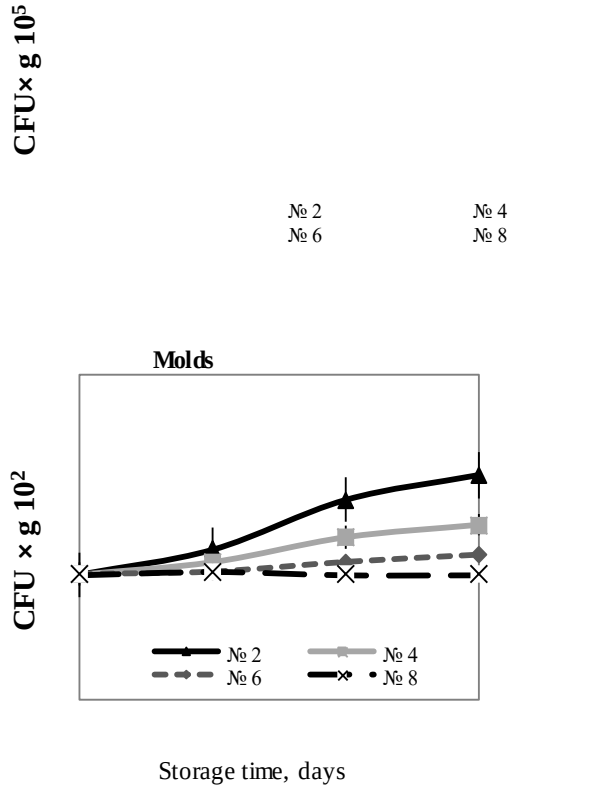
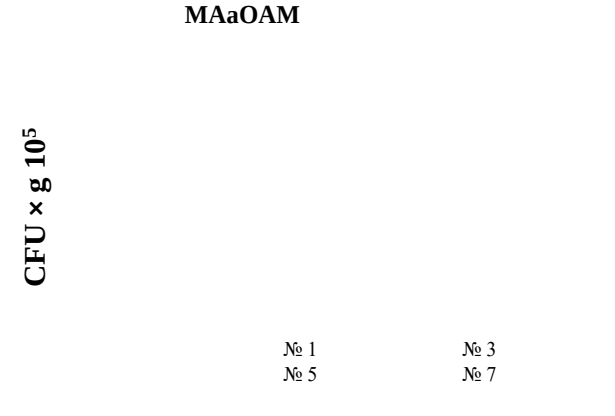


Fig. 2. Dynamics of the microbial population of the carrot roots surface during storage at t = +(25±1) °C (samples No 2, 4, 6, 8)

Fig. 3 shows the dynamics of the microbial population of the carrot roots surface during storage at t = +(2±1) °C (samples No 1, 3, 5, 7).



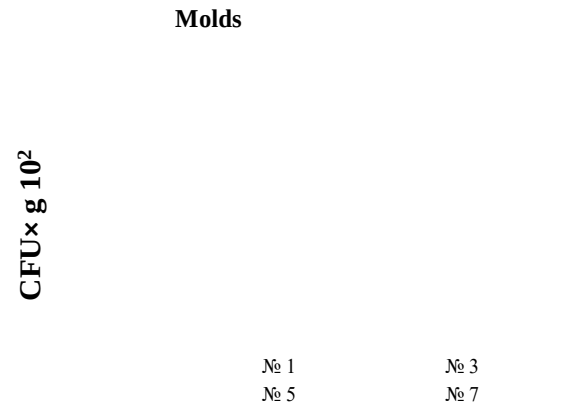


Fig. 3. Dynamics of the microbial population of the beet roots surface during storage at t = +(2±1) °C (samples No 1, 3, 5, 7)

Fig. 4 shows the dynamics of the microbial population of the beet roots surface during storage at t= + (25±1) °C (samples 2, 4, 6, 8).

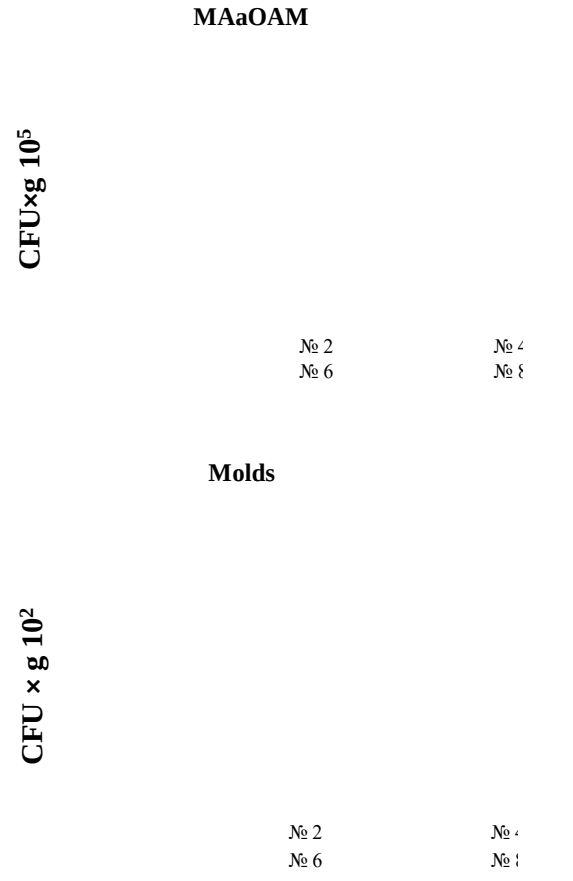


Fig. 4. Dynamics of the microbial population of the beet roots surface during storage at t = +(25±1) °C (samples No 2, 4, 6, 8)

IV. DISCUSSION

Conducted studies have shown that during the storage of garden carrots at t = +(2±1) °C (for 56 days), the amount of bacterial microflora (MAaOAM) of samples treated with a biological preparation (sample No. 3) decreased by the end of storage by 1.5 times compared to the reference sample, while this indicator for carrot subjected to the treatment by ELF-EMF (sample No. 5) decreased twice by the end of the storage. Complex treatment (sample No. 7) resulted in a decrease of MAaOAM by the end of storage by 2.5 times. The number of mold fungi in the samples treated with biological preparations decreased by the end of storage by 1.3 times compared to the reference. Samples subjected to treatment by ELF-EMF (sample No. 5) showed a decrease of MAaOAM by the end of storage by 1.6 times compared to the reference. The number of mold fungi of the samples subjected to complex treatment (sample No. 7) decreased twice by the end of storage compared to the reference sample. When storing garden carrots at t = +(25±1) °C (for 21 days), the number of MAaOAM on surface of the samples treated with a biological preparation (No. 4) decreased by the end of storage by 1.2 times compared to the reference, while samples subjected to treatment by ELF-EMF (No. 6) showed a decrease by 1.7 times. Complex treatment (No. 8) resulted in a decrease by 2.1 times. The number of mold fungi in the samples treated with biological preparation decreased by the end of storage by 1.2 times compared to the reference, while the number of MAaOAM for samples treated with ELF-EMF (No. 6) decreased by 1.4 times. Complex treatment (No. 8) resulted in a decrease in MAaOAM by 1.5 times.

When storing garden beets at t = +(2±1) °C (for 56 days), the number of MAaOAM in the samples treated with a biological preparation (No. 3), decreased by the end of storage by 1.1 times compared to reference; those subjected to ELF-EMF treatment (No. 5) showed the reduction by 1.4 times; those subjected to complex treatment (No. 7) revealed reduction by 1.5 times. The number of mold fungi in the samples treated by biological preparation decreased by the end of storage by 1.4 times compared to the reference; when treated by ELF-EMF – by 1.75 times; while after complex treatment – by 2.3 times.

When storing garden beets at t = +(25±1) °C (for 21 days), the number of MAaOAM of the samples subjected to treatment with a biological preparation (No. 4) decreased at the end of storage by 1.4 times compared to the reference samples; when treated by ELF-EMF (No. 6) – by two times, while after complex treatment (No. 8) – by 2.8 times.

The number of mold fungi in the treated samples decreased by 1.1 times by the end of storage compared to the reference; when treated by ELF-EMF – by 1.3 times; while after complex treatment – by 1.8 times.

V. CONCLUSION

Conducted studies have allowed revealing that the complex treatment of root crops of garden carrots and beets by biological preparation and ELF-EMF reduces the intensity of phytopathogens development more effectively in comparison to treatment by only biological preparations or only ELF-EMF treatment. The research revealed significant retardation in the growth rate of both bacterial and fungal pathogenic microflora during storage of root crops at different temperatures.

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