Application of air ionization in refrigerant storage of grapes refuse, boiled with starch syrup, in marshmallow technology

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Abstract. Experimental data on the change in the number and species composition of microorganisms in the atmosphere have been provided. These includes the changes on the walls of refrigeration chambers and on the surface of berries of the grape varieties Preobrazhenie and Livia when stored in a refrigerating chamber with a normal and ionized atmosphere. It was shown that the use of an ionized atmosphere with a concentration of positive aeroions of \(0.25 \times 1 \times 10^3\) and negative aeroions of \(0.11 \times 1 \times 10^3\) cm\(^3\) helped to reduce the overall microbiota contamination in a storage atmosphere by 94.7%. This happened on the surface of the walls and structures of the refrigerating chamber by 100% and on the surface of berries of the grape varieties Preobrazhenie and Livia by 95.6% and 97.1%, respectively.

1. Introduction
Grapes are valuable food products for dietary purposes, so it is desirable to extend the period of their consumption in fresh as long as possible. Grapes contain from 13.0 to 27.0% of sugars, represented mainly by glucose and fructose, 11 organic acids (malic, tartaric, citric, succinic, salicylic, formic, oxalic, etc.), 12 vitamins (A, C, P, B group, PP, etc.) and 48 different macro- and microelements [1, 2]. Grape skin is rich in phlobaphenes, catechins, anthocyanins and other phenolic compounds. Taking into account the high nutritional and biological value of grapes in the areas of its industrial production, the research is being conducted to develop a system for providing the population with fresh grapes all year round [3].

Specially created table (or table-technical) grape varieties are grown for seasonal and off-season consumption in fresh. They have an attractive appearance of bunches and berries, a fleshy consistency of the pulp, a harmonious taste, high transportability and keeping capacity [4]. In compliance with the agrotechnics of cultivation, harvesting time and storage technology such varieties as Shabash, Muscat Derbent, Moldova, Nimrang, Asma, Taifi Pink, Karaburnu and others can be stored up to 4 months or more [4-6].

The main losses of weight and quality of grapes and other juicy products during storage and transportation occur due to microbiological and physiological diseases [7-9]. The most harmful microbiological diseases of grapes during storage include gray rot or botrytiosis (Botritis cinerea), blue mold or Penicillium expansum (Penicillium expansum, P. glaucum), black mold or aspergillosis (Aspergillus niger), pink mold or Trichotecium roseum (Trichotecium roseum), black head mold or Rizopus (Rizopus nigerica) and other diseases [4, 6, 10].

To protect grapes from microbiological storage diseases, chemical and physical methods of control
are widely used. Among the chemical methods, the use of fundazole, sulfur dioxide, potassium or sodium metabisulfite, and other substances of chemical synthesis should be noted [4,9]. Among the physical methods controlled atmosphere (CA), dynamic atmosphere (DA), use of ultraviolet rays, saturation of storage chambers with ozone or high carbon dioxide, negative and/or positive aeroions have become widespread both independently and in combination with other techniques [1, 3-5, 8, 9, 11, 12].

To create an ionized atmosphere, polar or bipolar air ionizers are used in practical work. A distinctive feature of bipolar ionizers is the ability to produce aeroions of both polarities, while polar ionizers produce aeroions of the same polarity, usually negative. Atmospheric air ionization is widely used in apartments, car interiors, buildings, offices, cold storage rooms, fruit and vegetable storehouses, refrigerators in order to reduce the number of bacteria and fungi [1, 3, 12]. There is evidence of a positive effect of aeroions on increasing the shelf life of perishable products by reducing losses from fungal and bacterial diseases [8, 11].

The regulations for the use of an ionizer in the storage of perishable products should take into account the specific and varietal sensitivity of crops to the concentration of positive and negative aeroions. Therefore, in the development of technologies of their storage in the conditions of the ionized atmosphere, it is necessary to work out individual regulations of the device (the frequency of switching on the device and duration of its operation, power of the device, etc.). This will allow the maximum reduction in numerical and species composition of microflora in the storage atmosphere to preserve the original quality of products, including taste and aroma, as efficiently as possible [2, 6].

The aim of the research is to study the influence of positive and negative aeroions on the change in the numerical and species composition of microorganisms during the cold storage of grapes of the varieties Livia and Preobrazhenie.

2. Materials and methods

The research was carried out in the conditions of the Center for Collective Usage of scientific equipment "Selection of agricultural crops and technologies of production, storage and processing of functional and treatment-and-prophylactic products" of Michurinsk State Agrarian University. The objects of research were the grape varieties Preobrazhenie and Livia grown in OOO "Vinogradnaya Milya" (Limited Liability Company) of the Republic of Crimea.

Microbiological contamination was determined in the “Biophotonic” research problem laboratory. The assessment of the level of the infectious background of the atmosphere before and after storage was determined by the sedimentation method according to V. L. Omelyansky [8]. The essence of the method is the ability of microorganisms under the influence of gravity to settle on the surface of the nutrient growth medium in open Petri dishes. The calculation was made based on the fact that on 100.0 cm² of the surface of the nutrient medium in 5 minutes there are as many microorganisms as they are contained in 10.0 liters of air. When determining the total microbial contamination, Petri dishes with agarized potato-glucose medium were left open for 5 minutes. Sampling points were set according to the type of envelope: 4 points at the corners of the storage chamber (at a distance of 0.5 m from the walls) and the 5th point - in the center. Air samples were taken at a height of 1.6-1.8 m from the floor [12-17].

The quantitative and qualitative composition of the microbiota in the atmosphere of the chambers and localized on structures and fruits was determined by microbial culture of flushes from the test surface on a potato-glucose agarized medium, followed by recalculation per unit area. To create wet chambers, Petri dishes were taken, at the bottom of which a layer of filter paper was placed. The prepared Petri dishes were sterilized in a drying cabinet at 130°C for 3 hours. Before laying out the plant samples, the filter paper was moistened with sterile water. Petri dishes with samples were incubated at room temperature [18]. The grown colonies were analyzed and identified in accordance with generally accepted methods [18].

The microbiota from the fruit surface was washed off with sterile water. For this purpose, beakers with 100 ml of sterile distilled water were taken. The suspension of plant samples was placed in them
and shaken on a shaker. The flushes obtained were sown on agarized culture medium in Petri dishes [15]. In the process of exposure, the grown colonies were recorded, identified, and microscoped. The total microbial contamination was expressed by the number of CFU per 1 cm$^2$ of air or 1 cm$^2$ of the test surface.

Variants of the experiment:
- cold storage + normal atmosphere (NA) - t = 0...1°C, RH-95% (+/-1%);
- cold storage + ionizing air with an ionizer “Aeroclin” Italy (NA+IA) – t = 0...1°C, RH-95% (+/-1%), the concentration of positive aeroions – 0,25…0,48 ×10$^3$ cm$^3$, negative aeroions – 0,11…0,25×1×10$^3$ cm$^3$.

The concentration of positive and negative aeroions in the atmosphere of the cooling chambers was carried out using the device "Sapphire-3- M".

3. Results and discussion

Deterioration in the quality and loss of grapes during storage are mainly due to the development of infectious diseases (fungal and bacterial) or physiological, which occur without the participation of infection. Pathogens of microbiological diseases of grapes can get on the products during cultivation and harvesting, from the atmosphere of the refrigerator, from the walls, floors and other storage structures, or penetrate there with the container. It is known that the pathogens of some diseases (white rot, gray rot, anthracnose, fusarium, etc.) get on the products during the growing or harvesting period, and develop and affect it already during storage. Pathogens of other diseases infect and damage products only during the storage period. This group includes pathogens caused by mold fungi of the genera Penicillium, Aspergillus, Rizopus and bacteria of the genera Erwinia [14].

In the course of this work the qualitative and quantitative composition of the microbiota was determined in each case before and after storage in three places: in the atmosphere of cold storage, on the surface of the walls and structures of refrigerating chambers and on the surface of grape berries of studied varieties.

In compliance with the regulations for the preparation of storage facilities for the accepting of a new harvest, the numerical composition of all microorganisms, including pathogens of storage diseases, should be reduced to the minimum values. The preparation of the chambers for the accepting of the new harvest was carried out using the same technology. 25-30 days before putting the products for storage, the walls, floors, containers and other structures of the chambers were treated with a disinfectant solution, ventilated and UV lamps were installed in them for 90 minutes. After disinfection of the chambers, the quantitative and specific composition of microorganisms on the walls and in the atmosphere of the cold storage chambers was determined (Table 1).

| Table 1. Change in the total number of microorganisms during storage (shelf life of 75 days) |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Experiment                  | The atmosphere of the refrigerating chamber, CFU/ 1 cm$^2$ | Difference +/-, % | Walls of the refrigerating chamber, CFU/ 1 cm$^2$ | Difference +/-, % |
| Normal atmosphere          | Before storage | After storage    | Before storage | After storage    |
| Ionizing air                | 843            | 602              | 9865           | 3608            |
|                             | -28.6          | -63.4            |                |                 |
| Ionizing air                | 1686           | 90               | 4622           | 0               |
|                             | -94.7          | -100.0           |                |                 |

The experimental data obtained showed that before storage, the microbiological contamination of the atmosphere and the structures of the cold storage chambers significantly differed from each other. Thus, the total number of microorganisms in the atmosphere was higher in chamber No. 2 – 1686 CFU / cm$^3$. In the atmosphere of chamber No. 1, the total number of microorganisms was almost 2 times lower and amounted to 843 CFU/cm$^3$. On the walls of the refrigerator chamber, the total number
of microorganisms was higher in chamber No. 1 - 9865 CFU / cm$^2$. The total number of microorganisms in chamber 2 structures was significantly lower and amounted to 4,622 CFU/cm$^2$ (Table 1).

After 75 days of storage, there is a clear tendency to reduce the total microbial number (TMN), both on the surface of structures and in the atmosphere of cold storage chambers, both in the experimental and control versions. Thus, in the variant with NA, low temperatures (0...1$^\circ$C), even at high RH (94-96%), led to a decrease in the number of microorganisms in the storage atmosphere from 843 to 602 CFU/1 cm$^3$ (or 28.6%) and on the surface of walls and structures of cold storage chambers from 9865 to 3608 CFU/cm$^2$ (or 63.4%) (Table 1).

Experimental data have shown a high efficiency of positive and negative aeroions on the reduction of TMN in the atmosphere and, especially, on the surface of walls and structures of cold storage chambers. As a result of our research, the decrease in TMN in the atmosphere of the control chamber after 75 days of storage occurred by 28.6% (from 843 CFU/m$^3$ to 602 CFU/m$^3$), and in the ionization chamber - by 94.7% (from 1686 CFU/m$^3$ to 90 CFU/m$^3$). Ionization had an even more effective influence on reducing the number of microorganisms on the surface of walls and structures of cold storage chambers. Thus, if in the control version (NA), due to low temperatures, the number of microorganisms decreased by 63.4%, then in the version with ionization, due to the additional action of positive and negative aeroions, complete or 100% disinfection of the walls and structures of the cold chambers occurred.

The numerical and specific composition of microorganisms-pathogens on the surface of grape berries depends on many factors, the most important of which are varietal characteristics, agricultural cultivation techniques, precipitation, air humidity and temperature, protective measures against diseases, and others. The efficiency of grape storage will largely depend on the numerical and specific composition of microorganisms on the surface of the berries.

As a result of the conducted researches, it was found that the studied varieties differed significantly in the total number of microorganisms on the surface of the berries. The total microbiological contamination of the berries of the variety Livia was 157819.1 CFU / cm$^2$ and was 81771.3 CFU/cm$^2$ (or 52.0%) higher than that of the variety Preobrazhenie (76101.0 CFU/cm$^2$) (Table 2). It should be noted that the main part of the microorganisms on the surface of the berries were bacteria, which accounted for from 99.97 % in the variety Livia to 99.99% in the variety Preobrazhenie.

Table 2. Microbiological contamination of grape berries of the studied varieties before storage

| Variety       | Mycelial fungi CFU/cm$^2$ | %     | Bacteria CFU/cm$^2$ | %     | Total, CFU/cm$^2$ |
|---------------|--------------------------|-------|---------------------|-------|-------------------|
| Livia         | 53.2                     | 0.03  | 157819.1            | 99.97 | 157872.3          |
| Preobrazhenie | 10.1                     | 0.01  | 76090.9             | 99.99 | 76101.0           |

Mycelial fungi were found on the surface of the berries of the studied varieties in relatively small amounts from 10.1 CFU / cm$^2$ in the Preobrazhenie variety to 53.2 CFU/cm$^2$ in the Livia variety, which was 0.01 and 0.03% of the total number of microorganisms, respectively (Table 2). Representatives of the genera Botrytis, Cladosporium, Penicillium, Aspergillus, Mucor, and Alternaria were found from micellial fungi (Figure 1).
Figure 1. Mycelial fungi detected on the surface of grape berries: a) conidia of the Botrytis fungus (magnification ×80), b) conidia of the Cladosporium fungus (magnification ×640), c) conidia of the Alternaria fungus (magnification ×640)

During storage, the numerical and specific composition of microorganisms on the surface of grape berries of the studied varieties changed in different ways. As for the bacteria, the studied varieties in all storage variants had a decrease in their numerical composition. Thus, during 75-day cold storage under normal conditions, the number of bacteria decreased from 76090.9 to 70299.1 CFU/cm$^2$ (or by 7.6%) in the Preobrazhenie variety and from 157819.1 to 48803.4 CFU/cm$^2$ (or by 69.1%) in the Livia variety (Table 3).

Table 3. Quantitative and relative changes in the microbiological contamination of grape berries during storage (shelf life of 75 days)

| Variants       | Before storage. | Total. | After storage. | Change |
|----------------|-----------------|--------|----------------|--------|
|                | CFU/cm$^2$      | CFU/cm$^2$ | CFU/cm$^2$ | CFU/cm$^2$ |
|                | fungus | bacteria | fungus | bacteria | |
| Livia          | 53.2   | 157819.1 | 15782.3 | 8.5 | 48803.4 | 4881.9 | -69.1 |
| Preobrazh.     | 10.1   | 76090.9  | 76101.0 | 25.3 | 70299.1 | 70324.4 | -7.6 |
| Livia          | 53.2   | 157819.1 | 15782.3 | 9.0 | 4559.3  | 4568.3 | -97.1 |
| Preobrazh.     | 10.1   | 76090.9  | 76101.0 | 37.2 | 3251.4  | 3288.6 | -95.6 |

The magnitude of the decrease in the numerical composition of bacteria on the surface of the grapes of the studied varieties depended on the storage conditions and was maximum in the version with the use of ionization. Thus, the numerical composition of bacteria on the surface of grape berries in the variant with ionization decreased by 97.1% in the variety Livia and 95.7% in the variety Preobrazhenie, which is 28.0 and 88.1% higher than in the control variant, respectively.

The variation in the number of fungi during storage in our experiments was more influenced by varietal differences than by storage conditions. Thus, the numerical composition of fungi on the surface of the berries of the variety Livia after storage decreased from 53.2 to 8.5 CFU/cm$^2$ (or 84.0%) under normal atmospheric conditions and to 9.0 CFU/cm$^2$ (or 83.1%) under ionization conditions. In the Preobrazhenie variety, the numerical composition of fungi on the surface of berries after storage increased from 10.1 to 25.3 CFU/cm$^2$ (or by 150.5%) when stored under normal conditions and to 37.2 CFU/cm$^2$ (or 268.3%) when stored in the version with air ionization (Table 3).

A decrease in the TMN on the surface of the berries was common to the studied varieties in all storage versions. Thus, in the control variant, the decrease in this index was 7.6% in the Preobrazhenie variety and 69.1% in the Livia variety. The use of ionized atmosphere contributed to a decrease in the
total microbiota contamination of grape berries in the Preobrazhnenie and Livia varieties by 95.6% and 97.1%, respectively (Table 3).

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