Environmentally friendly technology for storing organically grown apples

M D Nazarko¹, G I Kasyanov², A A Zaporozhsky² and A V Kirichenko¹
¹Chair of Bioorganic Chemistry and Technical Microbiology, Kuban State Technological University, Krasnodar, Russia
²Chair of Technology of Food of Animal Origin, Kuban State Technological University, Krasnodar, Russia
E-mail: nazarko_m@mail.ru

Abstract. The proposed technology is aimed at optimizing agro-ecological and biotechnological methods to guarantee safety of the harvest and quality of apple products with no toxicological effects on the environment and humans. The proposed technology for storing ecologically clean apples is improved by using a complex that implies spraying with phytopreparation and exposure to low-frequency electromagnetic field. Comparative assessment is performed for morphological, organoleptic, chemical and technological characteristics of apples, the prevalence of scab and the yield of the garden with chemical and integrated preliminary protection techniques. The effect of electromagnetic field of low frequency in the range from 18 to 100 Hz on the activity of the fruit surface microflora is evaluated. A computer program is developed and registered to be used directly for analytics and forecasting the quality of long-term storage of products.

1. Introduction
Despite the advances in traditional agriculture, namely fruit growing, in providing mankind with fruits and raw materials, global environmental problems (environmental pollution, accumulation of pesticides, toxic and carcinogenic compounds) largely determined by this type of activity need immediate measures. Appropriate protection of fruit crops and finally fruit products based on systems that reduce man-induced impact and the use of chemicals is of high relevance and can solve problems emerging in ecological gardening. The idea of preserving and developing a ‘green environment around people’ is implemented in the international plant protection program GREEN BELT. Our studies allow joining this program to preserve and increase the yield of fruits with a focus on the ecosystem and human health.

Currently, increasing biologization and greening of horticultural production make microorganism-based biological preparations a safe alternative to chemical pesticides. Recent interest of Russian producers in environmentally friendly products, and the development of organic gardens will cause an increased demand for safer biological products and technologies for both consumers and the ecosystem.

The Krasnodar Territory is the country’s leading agricultural region. Fruit growing is one of its major industries. The topic is relevant to the region since it is in line with the Krasnodar regional target program Development of Agriculture and Regulation of Markets for Agricultural Products, Raw Materials and Food (2015), which includes goals and objectives to increase requirements for the environmental safety of agricultural production. Practice implementation of the principles of
environmental safety brings the system to a state of balanced protection of the natural environment, humans, and economic components. Currently, agricultural enterprises are making attempts to avoid the use of chemical pest and disease control agents, not only due to the adverse effect on the beneficial biota, but also due to direct harm to human health. Specialists pay great attention to the development of organic gardening, which allows them to supply the market with high-quality products [1, 2]. The contribution of agriculture of the Russian Federation to the global market of organic products is only 0.2%, but it has enormous potential for development of production since more than 10 million hectares of land are available and suitable for these purposes. Sociologists have identified good prospects for extending the area for apple gardens and increasing the turnover of apples in the future until 2023 [3, 4]. The aim of this study is to develop a set of measures to optimize biotechnological and physical methods that guarantee safety of the yield of the organic garden and the quality of apple products with no toxicological impact on the environment and humans.

In order to reduce losses during fruit storage, a method of treatment with phytopreparation and low frequency electromagnetic field (LF EMF) was proposed [5]. The data available in technical literature show that the effectiveness of LF EMF for reducing microbial contamination on the fruit surface is much superior to chemical methods of bactericidal treatment.

The authors participated in the development of a computer program that can be used to assess the content of vitamin C and dry matter during long-term storage of apples [6].

2. Study objects and methods

The object of the study is apples of the Aidared variety, late ripening, grown using a microbial biological preparation against Alternaria and scab, with no chemical protection involved, in a 15-hectare garden of OOO Agrofirm Krasny Sad (Rostov region, Azovsky district, Krasny Sad settlement).

In the study, refractometric, spectroscopic, thermogravimetric, extraction-chemical, potentiometric reference, titrometric, and amperometric methods were used to evaluate such indicators as the content of vitamins C and P, monosaccharides, dry substances and moisture, the content of mineral impurities, and the content of water-soluble antioxidants, titratable acidity, and pectin substances in fruits. The indicators of apple quality and safety were assessed in accordance with GOST R 54697-2011 using conventional methods [7]. Microbiological studies were conducted in compliance with GOST 31904-2012, 10444.15-94, 26669-85 [8–10]. The number of molds was determined in accordance with GOST 10444.15-94 [9].

The apples were harvested at the picking maturity stage of the highest and first commercial grade, and then stored in conditions that are in line with the requirements of the current applicable standards. The fruits were placed in boxes, 20–25 kg per box. The storage temperature was 2 °C, and relative humidity was 92%.

A complex pretreatment of apples for storage was performed. Optimal concentrations of the phytopreparation were selected experimentally, and then apples were sprayed. An aqueous extract from a mixture of dry leaves and pericarp of walnut prepared by 1-2 hour infusion in saturated water was used as phytopreparation [11].

The storage room was pretreated with LF EMF. In further treatment, the EMF LF frequency was also experimentally selected.

At the beginning, in the middle and at the end of storage, the following examinations were performed:
- visual inspection of apples to describe the commercial quality characteristics and identify spoilage;
- determination of the total number of microorganisms on the apple surface;
- evaluation of the quantitative and qualitative composition of bacterial and fungal microflora.

Statistical processing was performed using the Statistica v.10 software. Smoothing and bicubic spline interpolation by means of 3D graphics were employed to obtain the dependences of the

---

2
quantitative composition of bacterial and fungal microflora on the indicators of medium modifications, and the graphic method was used to optimize the storage process [12].

3. Results and discussion
In the Southern Federal District, apple varieties such as Idared, Red Chief, Florina, Liberty, Semerenko, Golden, William’s Pride, Ranger, Granny Smith and others are mainly grown. Unfortunately, diseases (scab, powdery mildew, etc.) and pests of fruit crops cause great harm to gardening, as a result, the yield significantly reduces and its quality deteriorates. The most harmful disease of cultivated apple varieties is scab, which affects the leaves and fruits of trees and causes a 30-40% loss of the yield. Figure 1 shows the appearance of apples grown at OOO AF Krasny Sad.

![Figure 1. Appearance of apples grown at OOO AF Krasny Sad.](image)

The Idared variety is characterized by large flattened rounded fruit with a smooth surface, the main color is light green with a bright crimson blush covering almost the entire fruit.

Morphological, biochemical, organoleptic and technological characteristics were studied for apple samples of the new harvest. It should be noted that the averaged values obtained fully corresponded to those of the Idared apple variety. Table 1 presents a comparative assessment of the morphological characteristics of apples, the prevalence of scab and the yield of the garden with two protection options applied: chemical and biological.

| Garden treatment option | Apple weight, g | Apple diameter, mm | Scab prevalence | Scab development,% | Number of apples per tree, pcs | Yield, t/ha |
|-------------------------|-----------------|--------------------|-----------------|--------------------|-------------------------------|-------------|
| Chemical                | 179.1           | 80.4               | 40.7            | 10.7               | 15.1                          | 6.65        |
| Biological             | 192.0           | 81.0               | 36.0            | 6.2                | 20.2                          | 8.24        |

It should be noted that the indicators of weight, diameter, color, apple yield and the number of apples per tree were found to be higher in the garden treated with biological preparations. The results of the microbiological study also indicated an improved phytosanitary condition of trees and fruits, since the prevalence and development of scab decreased.

To inhibit the development of microorganisms causing spoilage, apples of picking maturity were sprayed with phytopreparation, packed in a standard container and stored in a refrigerator with temperature ranging from 0 to +4 °C and humidity of 85–95.
During the study, we investigated the impact of LF EMF for cold sterilization of apples. We used amplitude and frequency modulated electromagnetic field for treatment of the studied objects, and read the biosystem response. Figure 2 shows a block diagram of the set-up for LF EMF treatment of biological objects.

**Figure 2.** Block diagram of the set-up for treatment of biological objects with EMF of different frequency: 1) analog-to-digital converter, 2) single-chip microcomputer, 3) random access memory, 4) digital-to-analog converter, 5) coupling amplifier, 6) high frequency generator, 7) modulator, 8) power amplifier, 9) inductor.

It was found that LF EMF treatment of biological systems affects the properties of free and bound water at the energy level. The biomolecule polarization changes when an object enters a stochastic resonance. The structure of water changed by the electromagnetic field can lead to either increased activity of microorganisms or their complete degradation.

Figure 3 presents a diagram of a device for LF EMF treatment of apples.

**Figure 3.** Diagram of the device for generating low-frequency and high-frequency modulated oscillations.

Low-intensity EMF affects the permeability of cell membranes, changes the ionic composition and the course of oxidative processes in mitochondria. Thus, depending on the frequency of irradiation and exposure, the magnetotropism of biological objects stimulates or suppresses biological processes.

At the beginning of the physical experiments, the initial indicators of the microflora of apples grown using biopreparations only were as follows: the number of bacteria, \( N_{\text{bac}} = 3.1 \pm 0.6 \) thousand, the number of filamentous fungi, \( N_{\text{fungi}} = 1.7 \pm 0.12 \) thousand, and the amount of yeast, \( N_{\text{yeast}} = 2.6 \pm 0.18 \) thousand.

The proposed technology for determining effective LF EMF frequencies and exposure time was realized in two stages. At stage 1, using a LF EMF generator at a temperature of about 2 °C and a
Relative air humidity of about 92%, the apple storage room was treated with amplitude modulated EMF with a carrier frequency of about 20 kHz and a carrier frequency of modulating oscillations $w = 42–52$ Hz for $t = 10–40$ minutes. As a result, $t$ is treatment time, min, and $w$ is the frequency, Hz, at which the values of $N_{bac}/N_{bac~in}$, the number of bacteria, $N_{fungi}/N_{fungi~in}$, the number of fungi, $N_{yeast}/N_{yeast~in}$, and the amount of yeast, as a function of $t$ and $w$, reach their maximum.

The data of mathematical processing obtained at experiment stage 1 are presented in Figure 4. 3D graphics Statistica 10, smoothing and bicubic spline interpolation of microbiological data were used to construct the surfaces of dependences of the number of bacteria, micromycetes and yeasts on treatment parameters at the first step of stage 1. At the second step of stage 1, the constructed dependences made it possible to determine that

- the dependence $N_{bac}=N_{bac~(t, w)} \rightarrow \max$ of the number of bacteria subjected to EMF treatment with a frequency of 42–52 Hz, at $t = 35$ min, $w = 46$ Hz, when $N_{bac}/N_{bac~in} = 2.5$;
- the dependence $N_{fungi}=N_{fungi~(t, w)} \rightarrow \max$ of the number of micromycetes subjected to EMF treatment with a frequency of 42–52 Hz, at $t = 45$ min, $w = 46$ Hz, when $N_{fungi}/N_{fungi~in} = 3.5$;
- the dependence $N_{yeast}=N_{yeast~(t, w)} \rightarrow \max$ of the amount of yeast subjected to EMF treatment with a frequency of 42–52 Hz, at $t = 40$ min, $w = 46$ Hz, when $N_{yeast}/N_{yeast~in} = 2.9$.

Relative errors of the values $N_{bac}/N_{bac~in}$, $N_{fungi}/N_{fungi~in}$ and $N_{yeast}/N_{yeast~in}$ at stage 1 were estimated equal to 16%; 5.7% and 10%, respectively.
Figure 4. Surfaces of dependences on the treatment parameters $t$ and $w$ at stage 1: (a) the number of bacteria on the apple surface after EMF treatment, (b) micromycetes, (c) yeast, (d) $N_{1st}$ according to (1) and determination of the optimal parameters of treatment stage 1. Lines indicate the optimal values $N_{bac}/N_{bac}^{in}$, $N_{fungi}/N_{fungi}^{in}$, $N_{yeast}/N_{yeast}^{in}$ and $N_{1st}$ according to (1); dots • are experimental data of stage 1.

At the third step of stage 1, vector optimization was performed with respect to the maximum criteria $N_{bac}/N_{bac}^{in}$, $N_{fungi}/N_{fungi}^{in}$ and $N_{yeast}/N_{yeast}^{in}$. According to the results of statistical data processing at stage 1, the optimal frequency of EMF treatment was determined, $w = 46$ Hz; however, the optimal time of LF EMF exposure differed for different systematic groups of microorganisms: the time of exposure was 35 minutes for bacteria, 45 minutes for filamentous fungi, and 40 minutes for yeast. The results presented in Figure 4d were used to determine the optimal time from the linear convolution of the criteria $N_{bac}$, $N_{fungi}$ and $N_{yeast}$ using the equation:

$$K_{icm} = K_{icm}(t,w) = \frac{1}{3} \left( \frac{K_{bacterium}}{2.5 K_{bacterium}^{initial}} + \frac{K_{mushrooms}}{3.5 K_{mushrooms}^{initial}} + \frac{K_{yeast}}{2.9 K_{yeast}^{initial}} \right) \cdot 100\% \rightarrow \text{max} \quad (1)$$

The studies revealed a slight increase in the number of microorganisms as a result of exposure to EMF with a frequency of 46 Hz. Treatment within 37 minutes facilitated the growth of microorganisms.

At stage 2, after 24 hours, the treatment mode was changed to a frequency modulated EMF with a carrier frequency of 15 kHz and a modulating frequency $w = 38 – 40$ Hz for $t$ up to 12 hours. The problem was posed and solved to determine treatment time $t$, min (hour), and frequency $w$, Hz, at which the values of $N_{bac}/N_{bac}^{in}$, the number of bacteria, $N_{fungi}/N_{fungi}^{in}$, the number of fungi, $N_{yeast}/N_{yeast}^{in}$, and the amount of yeast, as a function of $t$ and $w$, reach their minimum. Experimental data of stage 2 are shown in Figure 5.
Figure 5. Surfaces of dependences on the treatment parameters $t$ and $w$ of stage 2: (a) the number of bacteria on the apple surface after EMF treatment, (b) micromycetes, (c) yeast, (d) $K_{st}$ according to (2) and determination of the optimal parameters of treatment stage 2. Lines indicate the optimal values $N_{bac}/N_{bac}^{in}, N_{fungi}/N_{fungi}^{in}, N_{yeast}/N_{yeast}^{in}$ and $N_{1st}$ according to (1); dots • are experimental data of stage 2.

3D graphics Statistica v.10, smoothing and bicubic spline interpolation of microbiological data were used to construct the surfaces of dependences of the number of bacteria, micromycetes, and yeasts on the treatment parameters at the first step of stage 2. At the second step of stage 2, the constructed dependences made it possible to determine that

- the dependence $N_{bac}=N_{bac}(t, w) \rightarrow \min$ of the number of bacteria subjected to EMF treatment at $t = 1300$ min, $w = 38$ Hz, when $N_{bac}/N_{bac}^{in} = 0.05$;
- the dependence $N_{fungi}=N_{fungi}(t, w) \rightarrow \min$ of the number of micromycetes subjected to EMF treatment at $t = 1400$ min, $w = 38$ Hz, when $N_{fungi}/N_{fungi}^{in} = 0.02$;
- the dependence $N_{yeast}=N_{yeast}(t, w) \rightarrow \min$ of the numerical composition of yeast subjected to EMF treatment at $t = 1600$ min, $w = 38$ Hz, when $N_{yeast}/N_{yeast}^{in} = 0.01$.

Relative errors of the values $N_{bac}/N_{bac}^{in}, N_{fungi}/N_{fungi}^{in}$ and $N_{yeast}/N_{yeast}^{in}$ at stage 2 were estimated equal to 40%; 25% and 50%, respectively.

At the third step of stage 2, vector optimization was performed with respect to the minimum criteria $N_{bac}/N_{bac}^{in}, N_{fungi}/N_{fungi}^{in}$ and $N_{yeast}/N_{yeast}^{in}$. As a result of statistical processing of the microbiological indicators obtained at stage 2, the EMF frequency of 38 Hz was found to be optimal. The optimal exposure time that suppresses development and has a detrimental effect on microorganism cells was different for bacteria, filamentous fungi and yeast and amounted to 1300, 1400 and 1600 minutes, respectively. The optimal time was determined from the linear convolution of
the criteria $N_{bac}$, $N_{fungi}$ and $N_{yeast}$, which is (%) higher the achievable maximum possible for all 3 criteria, using the equation:

$$K_{2m} = K_{2m}(t,w) = \frac{1}{3} \left( K_{bacterium} \frac{K_{bacterium}}{0.05 \cdot K_{initial}} + K_{mushrooms} \frac{K_{mushrooms}}{0.02 \cdot K_{initial}} + K_{yeast} \frac{K_{yeast}}{0.01 \cdot K_{initial}} \right) \cdot 100\% \rightarrow \min$$  \tag{2}

The surface graph for determining the optimal treatment parameters with frequency modulated EMF was determined using spline interpolation of the data obtained: $N_{2m} = N_{2m}(t,w)$ (Figure 5). The optimal time for reducing the number of all systematic groups of microorganisms was 1420 minutes.

Thus, the optimal frequencies of EMF treatment and their duration were calculated at two successive stages. This method can be used not only to inhibit fruit rotting, but also to suppress pathogenic microflora on the walls of containers and premises. The method of non-contact antimicrobial treatment of fruits and vegetables, including LF EMF treatment of apples, implies that the storage room is first treated with an amplitude modulated EMF, and then with a frequency modulated EMF. This technology can be recommended for practical use.

Table 2 provides data on the dynamics of microbiological and chemical-technological indicators of apples taken as a control. Their initial microflora is diverse in qualitative composition and is represented by bacteria, yeast and filamentous fungi, with phytopathogenic representatives among them. During storage, the number of microorganisms increased on average 10–30-fold, especially the number of micromycetes. After 4 months, the number of affected fruits exceeded 30%.

Table 2. Microbiological and chemical-technological indicators of apples grown using chemicals and stored without treatment.

| Storage duration, days | 0   | 90  | 120 |
|------------------------|-----|-----|-----|
| Number of damaged fruits, % | 0   | 24.5 | 42.6 |
| Yield of standard products | 100 | 75.0 | 56.0 |
| Bacteria, CFU/cm² | 57.0±0.42 | 95.0±0.56 | 300.0±9.7 |
| Micromycete, CFU/cm² | 9.6±0.15 | 25.0±0.5 | 63.0±4.4 |
| Yeast, CFU/cm² | 7.3±0.18 | 19.8±0.4 | 35.6±1.2 |
| Vitamin C, mg/100 g | 1.49±0.1 | 0.5±0.03 | 0.3±0.01 |
| Mass loss, % | 0 | 15.0 | 22.0 |

Table 3 provides data on microbiological and chemical-technological indicators of apples, which were grown using biological preparations and treated with phytopreparations for storage.

Table 3. Microbiological and chemical-technological indicators of apples grown using biological preparations and stored in the modified environment.

| Storage duration, days | 0   | 90  | 120 |
|------------------------|-----|-----|-----|
| Number of damaged fruits, % | 0   | 8   | 12  |
| Yield of standard products | 100 | 90.0 | 86.0 |
| Bacteria, CFU/cm² | 31.0±0.01 | 59.0±0.6 | 20.0±0.1 |
| Micromycete, CFU/cm² | 1.7±0.1 | 15.0±0.3 | 0.1±0.03 |
| Yeast, CFU/cm² | 2.6±0.02 | 20.0±0.2 | 0.18±0.02 |
| Vitamin C, mg/100 g | 3.74±0.2 | 3.22±0.2 | 2.90±0.1 |
| Weight loss, % | 0 | 1.1 | 2.4 |

It should be noted that the initial indicators of the microflora are lower for apples grown using biological preparations. Apples treated with phytopreparations showed a decreased number of bacteria and micromycetes, but after 4 months of storage, the microflora was observed to increase again. During storage, apples exhibited a gradual decrease in the vitamin C content and a decrease in weight.
However, these indicators were significantly lower than those in the control. This should be considered as a positive effect of phytopreparation and LF EMF treatment. Apples stored without treatment were mainly affected by fruit rot, and apples subjected to phyto-treatment exhibited gas injury and wilting.

4. Conclusions

To date, the storage of products instead of selling on the market aims to gain additional profit through either the prospective sale of products at beneficial market prices, or preservation of product quality, and in some cases, through the improvement of the product individual quality characteristics. Based on generalized practical experience in storing agricultural products and investigation of the market proposals, we have developed a set of physical and biological measures to protect apple fruit from phytopathogenic microflora that causes spoilage. The types of treatment used significantly reduced not only contamination of fruit by microorganisms, but also increased the shelf life and product quality.

Acknowledgment

The research was financially supported by the Russian Foundation for Basic Research and the Administration of Krasnodar Territory within the framework of scientific project #18-413-230013 r_a

References

[1] Doroshenko N T, Buzoverov A V, Chumakov S S and Reazanova L G 2013 Organic Gardening (Krasnodar: Kuban State Agrarian University)
[2] Pachkin A A, Vasilyeva L A, Yakovuk V A, Doroshenko T N, Niyazov O D and Balakhina O V 2016 Bulletin of Agrarian Science 1 44–57
[3] Nazarko M D, Lobanov V G, Kasyanov G I and Romanets I I 2019 Proceeding of International scientific and practical conference fifth technological way: prospects for development and modernization of the Russian agro-industrial complex (TFTS 2019)124–129
[4] Nikolaeva M A and Lebedeva T P 2016 Siberian trade and economic journal 2 106–109
[5] Kupin G A, Pershakova T V, Gorlov S M, Viktorova E P, Matvienko A N and Velikanova E V 2017 Investigation of the effect of processing fruits with electromagnetic fields of extremely low frequencies and biological products the loss of biologically active substances during storage Polythematic electronic journal KubSAU 132 1111-21
[6] Nazarko M D, Kasyanov G I, Lobanov V G, Inochkina E V, Kirichenko A V and Romanets I I 2019 Certificate of state registration of the Computer Program № 2019660701 dated 12.08.2019 A program for building a regression model for assessing the chemical and technological indicators of apple fruits during storage
[7] GOST R 54697-2011 2013 Fresh apples, sold in retail outlets. Technical conditions (Moscow: Standardinform)
[8] GOST 31904-2012 2014 Food products. Sampling methods for microbiological testing (Moscow: Standardinform)
[9] GOST 10444.12-2013 2014 Microbiology of food and animal feed. Methods for the detection and counting of yeast and molds (Moscow: Standardinform)
[10] GOST 10444.15.94 2010 Food products. Methods for determining the number of mesophilic aerobic and facultative anaerobic microorganisms (Moscow: Standardinform)
[11] Nazarko M D, Kasyanov G I, Kirichenko A V, Ovchinnikova E I, Romanets I I and Erofeeva D A 2019 Method of producing a preparation for processing vegetables and fruits Patent for invention RU № 2710172
[12] Koneva M S, Rudenko O V, Usatikov S V, Bugaets N A, Tamova M Yu, Fedorova M A and Mogilnyi M P 2018 Journal of pharmaceutical sciences and research 10(2) 381–390