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Development of a scientific concept of industrial storage systems for environmentally safe apples

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ABSTRACT

The research project has developed and justified the storage modes of apples in a modified gas environment by creating an isolated "closed loop" of high-pressure polyethylene; the expediency of creating highly efficient technologies for storing fresh fruits in a controlled atmosphere, in bioactive bactericidal packages and by creating microfilm on the surface of fruits has been confirmed. The prospects of using a progressive method of storing fruits in a modified gas atmosphere by creating an isolated "closed circuit" in a separate refrigerating chamber without using expensive equipment (in normal and subnormal gas environments) are proved. New technologies have been developed for storing apple fruits susceptible to infectious and physiological diseases based on improved storage methods with minimal losses. The consumption rates of *Phytosporin-M* for the surface treatment of fruits were determined and optimized to control the intensity of biochemical and microbiological processes during storage. The modes and technologies of post-harvest fruit processing with the *Phytosporin-M* biopreparation have been substantiated.

Keywords: apple, storage, gas environment, closed loop, bioactive coatings, bactericidal packaging

INTRODUCTION

A significant part of the grown fruit products is lost during storage. For some types, these losses often reach 30% and sometimes 50% [1], [2]. Therefore, developing highly efficient storage technologies is an urgent task today [3], [4], [5], [6]. All methods of storing fruits in a modified gas atmosphere can be classified according to the type of medium used, the method of management, methods of creating the medium, etc. [7], [8]. The history of developing methods for storing fruit and vegetable products shows that atmospheric air and its components are used in most cases. Storage in artificially created gases (for example, ozone) has not found wide application in practice due to their high cost or low efficiency (for fruits) [9], [10], [11].

Based on the gas composition of atmospheric air and depending on the type of environment created on its basis, we conducted research on the storage of fruits and vegetables:

- in a controlled atmosphere (CA);
- in bioactive bactericidal packaging;
- with a surface coating of fruits with biologically active preparations.

The advantages of storing fruit products in a controlled atmosphere (CA) are well known. However, such storage does not replace storage in the refrigerator (its temperature is usually 3 – 5 °C higher than in the refrigerator). The storage of fruit products in the CA slows down the processes of maturation and overripening, as a result of which its shelf life is lengthened, the quality and yield of marketable products are increased, immunity is preserved, microbial spoilage and physiological damage are reduced [12], [13], [14]. Biological oxidation (respiratory gas exchange or respiratory activity) is at the center of physiological and biochemical processes during fruit ripening. All physiological and biochemical changes in fruit products are made due to the energy released during breathing [15], [16], [17].

In plant cells, cytochrome oxidase, polyphenol oxidase, ascorbate oxidase and flavin enzymes can perform the functions of O₂ activators at the final stages of respiration. With a change in the concentration of O₂ and CO₂, the proportion of participation of individual oxidases in the respiration process changes. At very low concentrations of O₂, cytochrome oxidase and peroxidase activity increases sharply, and the activity of flavoprotein enzymes decreases. The middle position is occupied by polyphenol oxidase and ascorbate oxidase. High concentrations of CO₂ (7 – 10%) block respiration due to a sharp decrease in carboxylase, cytochrome oxidase, pyruvate dehydrogenase, etc., and also disrupt many other processes of fruit metabolism [18], [19], [20]. The composition of the gaseous medium (CO₂ and O₂) and temperature are important factors regulating the rate of biological oxidation in plant cells. Therefore, data on the effect of individual components of the gaseous medium on the rate of fetal respiration are of particular interest [21], [22]. The transition of one phase of development (maturation) to another (overripe) is characterized by a change in the predominance of certain enzymatic systems that catalyze this process during respiration [23], [24], [25], [26].

Therefore, developing a method for storing fruits in a modified gas atmosphere is promising and relevant by creating an isolated "closed loop" and a system for reducing fruit losses based on complex post-harvest treatments with biologically active preparations.

Scientific Hypothesis

Creating an isolated "closed loop" and carrying out complex post-harvest treatments of apples with biologically active preparations can ensure a reduction in fruit losses during storage.

MATERIAL AND METHODOLOGY

Samples

The samples were the fruits of promising varieties of domestic and foreign breeding, grown in the soil-climatic conditions of the Krasnodar Territory (Russia): Jonathan, Reinette Simirenko, Idared and Korey.

Chemicals

Ethanol, Sodium carbonate, Sodium phosphoric acid, Sodium sulphate, Potassium ferruginous, Zinc acetic acid, Zinc sulphate, Lead acetic acid, Nitric acid, Sulfuric acid, Hydrochloric acid, Oxalic acid, Sodium hydroxide, Potassium permanganate, Phenolphthalein. All chemicals above were purchased by LenReactive LLC (Sants Petersburg, Russia) and were of analytical grade quality).

Apple samples were treated with Phytosporin-M preparation (BashInkom Inc., Russia).

Animals and Biological Material

The apple variety *Malus domestica* (var. Idared, Korey, and Jonathan).

Instruments

GHP-75 gas analyzer (Alta LLC, Stavropol, Russia), FT - 372 penetrometer with plunger (Alitus LLC, Moscow, Russia), Abbe NAR-4T Refractometer (ATAGO Inc., Tokyo, Japan), UV-1800 spectrophotometer (Shimazy Inc., Tokyo, Japan), Climate camera BINDER KBW 240 (BINDER GmbH, Tuttlingen, Germany).

Laboratory Methods

Analytical and experimental studies were based on the macroscopic theory of gases, modern ideas about the physiological state of storage objects, mass transfer theory, and various fruits' storage technologies.

Fruit quality assessment was conducted according to the "Program and methodology of variety study of fruit, berry and nut crops" [27]. The fruits for analysis were selected at removable maturity. During the technical analysis, the mass, the size of the fetus, and the shape index, i.e., the ratio of height and diameter, were measured. Biochemical studies were carried out in 3-5-fold repetition in the biochemical laboratory of fruit storage and processing. The hardness of the apple pulp was determined by an FT - 372 penetrometer with a plunger with a diameter of 11 mm; tasting evaluation – according to GOST 8756.1-79 [28]; the chemical composition of apples was determined by methods generally accepted in fruit biochemistry: soluble dry substances – by the refractometric method (GOST 28562-90) [29]; sugars – by the Bertrand spectrophotometric method (GOST 8756.13-87) [30]; titrated acidity – by titration with 0.1N NaOH solution (GOST 25555.0-82) [31]; vitamin C – with potassium iodate (GOST 24556-89) [32]; pectin substances – by titrometric method (GOST 29059-91) [33]; alcohols and aldehydes – by spectrophotometric carbazole method [34], polyphenolic substances – by vanillin method [34]. Natural decline, spoilage, total losses, and output of marketable products were determined according to recommendations of GOST 34314-2017 [35].

Description of the Experiment

Sample preparation: To prepare the apples for the experiment, the samples were packed in a polyethylene film with a thickness of 40 – 120 microns or lowered into a solution of the drug Phytosporin-M to form an active nanofilm.

Number of samples analyzed: 100 in each experimental group.

Number of repeated analyses: 3.

Number of experiment replication: 2.

Design of the experiment:

Analytical and experimental studies were based on the macroscopic theory of gases, modern ideas about the physiological state of storage objects, mass transfer theory, and various fruits' storage technologies. To prepare the apples for the experiment, the samples were packed in a polyethylene film with a thickness of 40 – 120 microns, or lowered into a solution of the drug Phyto sporin-M to form an active nanofilm (Figure 1).



Figure 1 Experimental samples of apple.

Fruit quality assessment was conducted according to the "Program and methodology of variety study of fruit, berry and nut crops" [27]. The fruits for analysis were selected at removable maturity. During the technical analysis, the mass, the size of the fetus, and the shape index, i.e., the ratio of height and diameter, were measured. Biochemical studies were carried out in 3-5-fold repetition in the biochemical laboratory of fruit storage and processing. The hardness of the apple pulp was determined by an FT-372 penetrometer with a plunger with a diameter of 11 mm; tasting evaluation – according to GOST 8756.1-79 [28]; the chemical composition of apples was determined by methods generally accepted in fruit biochemistry: soluble dry substances – by the refractometric method (GOST 28562-90) [29]; sugars – by the Bertrand spectrophotometric method (GOST 8756.13-87) [30]; titrated acidity – by titration with 0.1N NaOH solution (GOST 25555.0-82) [31]; vitamin C – with potassium iodate (GOST 24556-89) [32]; pectin substances – by titrimetric method (GOST 29059-91) [33]; alcohols and aldehydes – by spectrophotometric carbazole method [34], polyphenolic substances – by vanillin method [34]. Natural decline, spoilage, total losses, and output of marketable products were determined according to recommendations of GOST 34314-2017 [35].

Statistical Analysis

Statistical processing of experimental data was carried out using the NSR.BASE Excel software and analysis of variance using Student's criterion with confidence probability ($\alpha = 0.95$) [36]. Statistically significant values were assumed at $p < 0.05$. Values with $p > 0.05$ were not taken into account in the experiment.

RESULTS AND DISCUSSION

Our research has shown that under optimal storage conditions in CA, the respiration of apples of various varieties proceeds evenly and slowly. So, after 5 – 7 months, the respiration rate of Jonathan and Reinette Simirenko apples was 55 – 77% compared to fruits stored under normal conditions (control). A similar trend was previously established by other researchers in the study of different varieties and fruits [37], [38], [39].

In the most long-stored variety of Korey apples, the initial state is characterized by the lowest respiratory intensity (2.3 mg/kg/h), which is slightly higher in Idared apples (2.7 mg/kg/h), but different periods of climacteric rise. In the control storage variant, the climacteric rise of respiration in the Korey variety began after 5.5 months, in Idared after 4 months. When stored in a "closed loop" with increased CO₂ content, the climacteric rise in respiration was weakly pronounced and stretched over time, which corresponds to the results of other researchers [40], [41].

According to Calegario et al. (2001), the timing of the climacteric rise of fetal respiration and the steepness of the fall correlates with the rate of entering the gas regime in a "closed loop" and with the rate of biochemical processes [42].

To clarify the respiratory gas exchange during the storage of apples in a "closed loop", the gas composition was determined using the GHP-75 gas analyzer. The results obtained are presented in Table 1.

Table 1 The content of O₂ and CO₂ in the "closed loop" ($p < 0.05$).

Apple variety	The composition of the air in a "closed loop" during storage, %							
	1 month		2 months		4 months		7 months	
	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂	O ₂	CO ₂
Idared:								
p/e film 120 μm	14.5	6.5	13.0	8.0	12.0	9.0	nitrogen purging or perforation	
p/e film 40-60 μm	18.0	3.0	14.2	6.8	12.8	8.2	12.8	8.2
Korey								
p/e film 120 μm	15.2	5.8	14.0	7.0	12.5	8.5	nitrogen purging or perforation	
p/e film 40-60 μm	19.0	1.0	15.3	5.7	13.5	7.5	13.0	8.0

From the data in Table 1, it can be seen that when storing fruits in a "closed loop", the gas composition changes in the direction of increasing the concentration of carbon dioxide. The degree of change in the gas environment depends on the varietal characteristics of the fruits [43], [44]. According to our data, significant changes in the gas composition in the package occur during the first month of storage: a decrease of O₂ from 1.5 to 3.7% and an increase of CO₂ from 1.5 to 4.7%. For further storing, there is a gradual decrease in oxygen and an increase in carbon dioxide. In the contour of a 40 – 60 μm film, the steady-state mode (due to fruit respiration) is stable until the end of storage (7 months). In a "closed loop" of a 120 μm film, the gas medium was also created as a result of fruit respiration, but after 4 months of storage, it was maintained by blowing or opening the valve.

Biochemical quality indicators are presented in Table 2. After 7 months of storage in a "closed loop" (120 μm), Fruits had good preservation of dry substances and sugars and a sourer taste than fruits stored with free access to air. At the same time, the higher acidity of the fruit is mainly due to the content of malic acid, which is explained by the inhibitory effect of carbon dioxide on the intensity of respiration [45], [46].

Changes in the mass fraction of ascorbic acid in apples under the influence of a gaseous medium differed little from control samples. This confirms the assumption of Sapei and Hwa (2014) that the dynamics of vitamin C is less dependent on the composition of the medium and a greater correlation with the temperature regime of storage [47]. The increased content of carbon dioxide and the reduced oxygen content in the medium contributed to the ripening of the fruits to a lesser extent, as evidenced by the data on the content of alcohol and acetaldehydes after 7 months of storage. The mass fraction of them, when stored in a "closed loop" was minimal compared to the control (Table 2).

It should be noted that apples stored in a modified gas atmosphere in p/e films of 40 and 60 μm also had good storage results in terms of commodity and chemical quality indicators (in comparison with the control) but were inferior to apples in a "closed loop" (120 μm) in all indicators.

Processing of apples of the Idared variety before laying for storage with the biological preparation *Phytopsporin-M* (40, 60 μm) also positively affected the quality of storage, reducing losses from microbial spoilage and reducing the gap in the yield of marketable products between fruits stored in a "closed loop" (120 μm).

Table 2 Biochemical indicators of apple fruit quality after 7 months of storage in a modified gas environment ($p < 0.05$).

The variety of apples with the method of storage	Soluble dry substances, %	Total sugars, %	Total acidity, %	Alcohol, %	Aldehydes, % mg/dm ³	Pectin substances, %		Polyphenolic substances, mg %	Vitamin C, mg %
						instant pectin	Proto-pectin		
Korey (when laying for storage)	13.0	12.0	0.33	–	–	0.29	0.62	240.0	7.0
Korey control (without treatment) storage in containers	12.2	10.9	0.22	0.150	11.65	0.26	0.40	190.0	4.7
Korey in p/e film 40 μm	12.4	11.2	0.23	0.091	11.42	0.25	0.44	190.0	5.0
Korey in p/e film 60 μm	12.5	11.4	0.24	0.069	9.64	0.23	0.45	196.0	5.5
Korey in p/e film 120 μm ("closed loop")	12.7	11.7	0.26	0.058	6.90	0.22	0.50	220.0	5.9
Idared (when laying for storage)	12.0	10.8	0.58	–	–	0.34	0.50	150.0	8.8
Idared control (without treatment) storage in containers	10.3	9.8	0.36	0.173	17.35	0.13	0.27	123.3	6.9
Idared in p/e film 40 μm	10.4	9.8	0.38	0.104	14.62	0.13	0.30	124.5	7.4
Idared in p/e film 60 μm	10.8	10.0	0.38	0.092	9.00	0.12	0.32	126.3	7.4
Idared in p/e film 120 μm ("closed loop")	11.3	10.4	0.44	0.068	7.80	0.13	0.42	131.5	7.7

From the results obtained, summarized in Table 3, it can be seen that the method of storing apples in an "isolated circuit" gives a pronounced positive effect since losses are significantly reduced (from 4.5% to 0.4%) from weight loss and, very importantly, from microbiological spoilage from 4.6% to 0.8% and physiological diseases, which guarantees the safety of nutrients. At the same time, the yield of commercial varieties increased by 7 – 8.5%, depending on the variety.

When storing apples in a modified gas atmosphere, the following requirements must be observed: to store fruits with high-quality indicators at optimal maturity; to load chambers with products homogeneous in keeping quality and immediately after harvesting; varieties susceptible to sunburn must be treated with antioxidants or biological preparations [48], [49]. Failure to comply with these conditions reduces the shelf life, losses, and quality of fruits.

Thus, studies have shown that our proposed method of storing apples in an isolated "closed loop" using high-pressure polyethylene with a film thickness of 120 μm, without the use of expensive equipment and materials for sealing chambers, allows us to achieve storage results close to storing apples in a controlled atmosphere [50], [51], [52]. Also, we used existing fruit storage capacities and parts of them while maintaining constant access to the chambers, which is very important for farms that grow small volumes of apples and cannot build separate refrigeration facilities [53], [54].

Infectious and physiological diseases of fruits annually cause huge economic damage to the industry [55, 56]. This problem is solved by treatment with harmless and effective drugs that reduce the microbiological load in food technologies.

Apples of winter varieties were used: Idared, Korey, Jonathan. A prolonged-acting microbiological fungicide with anti-stress properties, *Phytosporin-M*, was used for surface treatment. The apple surface treatment was carried out according to the method proposed by Gvozdenko et al., 2022 [57]. The processed apples were dried and sent to refrigerated storage for 7 days. To determine the optimal consumption rates of the *Phytosporin-M* fungicide for post-harvest processing of raw materials, a solution of 1 – 1.5 liters per ton was used.

Table 3 Commercial quality of apples when stored in a modified gas environment ($p < 0.05$).

The variety of apples with the method of storage	For 4 months of storage				For 7 months of storage			
	Natural decline, %	Spoilage, %	Total losses, %	Output of marketable products, %	Natural decline, %	Spoilage, %	Total losses, %	Output of marketable products, %
Idared control, storage in containers	2.4	1.4	3.8	96.2	4.9	3.7	8.6	91.4
Idared in p/e film 40 μm (treated with a bactericidal preparation)	0.0	0.0	0.0	100.0	0.7	3.1	3.8	96.2
Idared in p/e film 60 μm (treated with a bactericidal preparation)	0.0	0.0	0.0	100.0	0.6	2.4	3.0	97.0
Idared in p/e film 120 μm	0.0	0.0	0.0	100.0	0.5	1.1	1.6	98.4
Korey control, storage in containers	2.1	2.7	4.8	95.2	4.2	5.1	9.3	90.7
Korey in p/e film 40 μm	0.0	0.0	0.0	100.0	0.6	3.9	4.4	95.6
Korey in p/e film 60 μm	0.0	0.0	0.0	100.0	0.5	2.0	2.5	97.5
Korey in p/e film 120 μm	0.0	0.0	0.0	100.0	0.4	0.7	1.1	98.9

The results of the optimal solution concentration were judged by the commodity assessment of the quality of raw materials during storage: the presence of rot, diseased, and sprouted specimens. The experimental batch of plant raw materials received the best commodity rating and was studied according to organoleptic, biochemical, and microbiological quality indicators.

Comparative data on the quality of fruits treated with a solution of *Phytopsporin-M* and control (without treatment) are presented in Table 4, Table 5, and Figure 2 and Figure 3.

Table 4 Commercial quality of apples treated with *Phytopsporin-M* during storage at 0 +2° C ($p < 0.05$).

The variety of apples with the method of storage	For 4 months of storage				For 7 months of storage			
	Natural decline, %	Spoilage, %	Total losses, %	Output of marketable products, %	Natural decline, %	Spoilage, %	Total losses, %	Output of marketable products, %
Korey control, storage in containers	2.1	2.7	4.8	95.2	4.2	5.1	9.3	90.7
Korey, treated with a bactericidal preparation, storage in containers	1.4	1.0	2.4	97.6	3.0	2.3	5.3	94.7
Idared control, storage in containers	2.4	1.4	3.8	96.2	4.7	2.9	7.6	92.4
Idared, treated with a bactericidal preparation, storage in containers	1.8	1.1	2.9	97.1	3.7	2.0	5.7	94.3
Jonathan control, storage in containers	3.5	2.0	5.5	94.5	7.8	4.6	12.4	87.6
Jonathan, treated with a bactericidal preparation, storage in containers	2.5	1.2	3.7	96.3	5.6	2.6	8.2	91.8

According to research results, pretreatment with *Phytopsporin-M* provides the best yield of marketable products, minimal microbial spoilage (Figure 2 and Figure 3), and loss of biologically active substances (Figure 4).

To preserve the natural stability of fruits and prolong their shelf life, preventing the intensive accumulation of alcohol is of great importance [58]. At the next stage of the experiment, apple samples were packed in a polyethylene film with a thickness of 40 – 120 microns or lowered into a solution of the drug Phytosporin-M to form an active nanofilm [57], [59], [60], [61]. According to our data (Table 5), the alcohol content in processed fruits by the end of storage (7 months) is 1.5 times lower than in the control.

Table 5 Biochemical quality indicators of apple fruits treated with *Phytosporin-M* after 7 months of storage ($p < 0.05$).

The variety of apples with the method of storage	Soluble dry substances, %	Total sugars, %	Total acidity, %	Alcohol, %	Pectin substances, %		Polyphenolic substances, mg %	Vitamin C, mg %
					instant pectin	Proto-pectin		
Korey (when laying for storage)	13.0	12.0	0.33	–	0.29	0.62	240.0	7.0
Korey control (without treatment) storage in containers	12.2	10.9	0.22	0.150	0.26	0.43	190.0	4.7
Korey processed Phytosporin-M (in containers)	12.5	11.5	0.29	0.093	0.24	0.45	215.0	5.5
Idared (when laying for storage)	12.0	10.8	0.58	–	0.34	0.50	150.0	8.8
Idared control (without treatment) storage in containers	10.3	9.9	0.38	0.173	0.13	0.27	123.3	6.9
Idared processed Phytosporin-M (in containers)	11.0	10.2	0.42	0.114	0.12	0.30	133.8	7.5
Jonathan (when laying for storage)	12.5	11.5	0.60	–	0.50	0.38	170.0	8.0
Jonathan control (without treatment) storage in containers	10.2	10.0	0.39	0.185	0.16	0.22	130.0	5.9
Jonathan processed Phytosporin-M (in containers)	11.2	10.9	0.44	0.125	0.13	0.25	145.0	6.4

The microbiological studies of apple fruits during storage fully confirm the advantages of storing fruits with their pretreatment with *Phytosporin-M* fungicide (Figure 4). This technology makes it possible to reduce losses from microbial spoilage by 2 times for the Korey and Jonathan varieties and by 1.6 times for the Idared variety (Figure 2 and Figure 3).

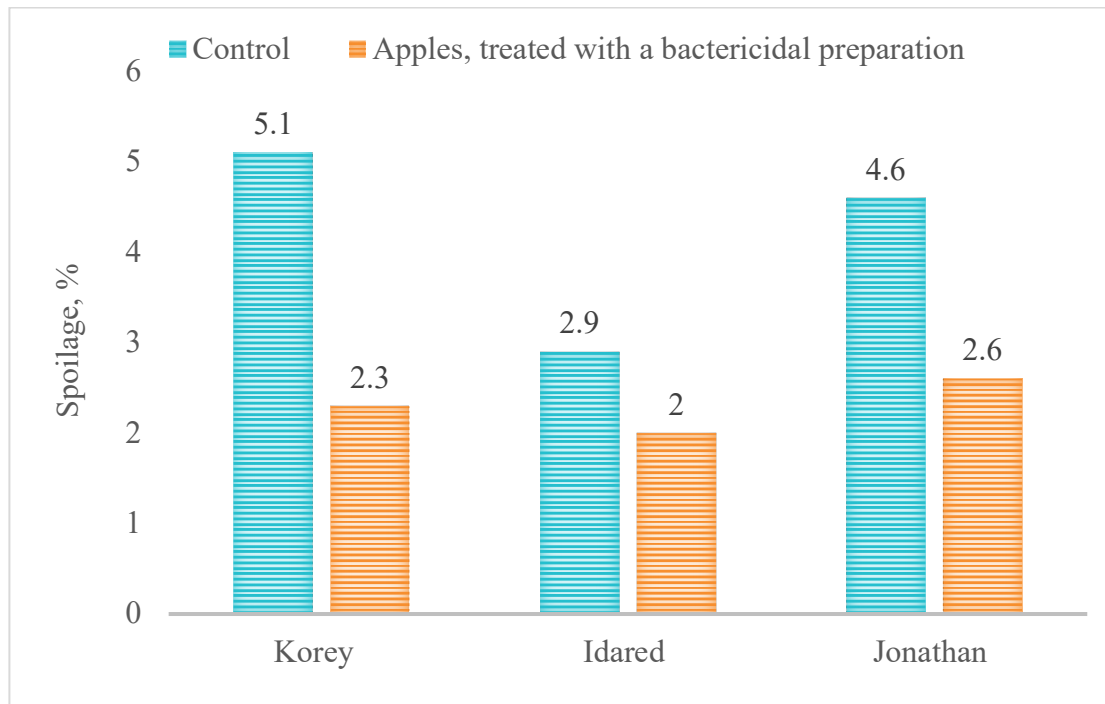


Figure 2 The effect of processing apples with a biological preparation on the amount of microbiological spoilage during 7 months of storage ($p < 0.05$).

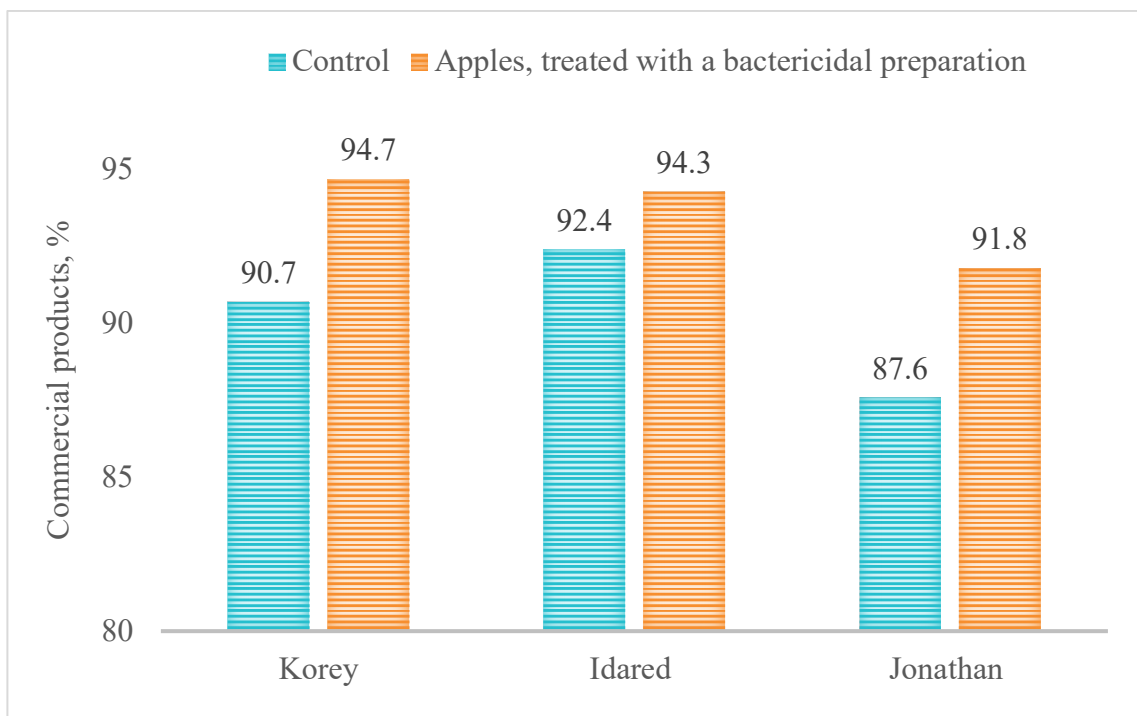


Figure 3 The effect of processing apples with a biological product on the yield of marketable products during 7 months of storage ($p < 0.05$).

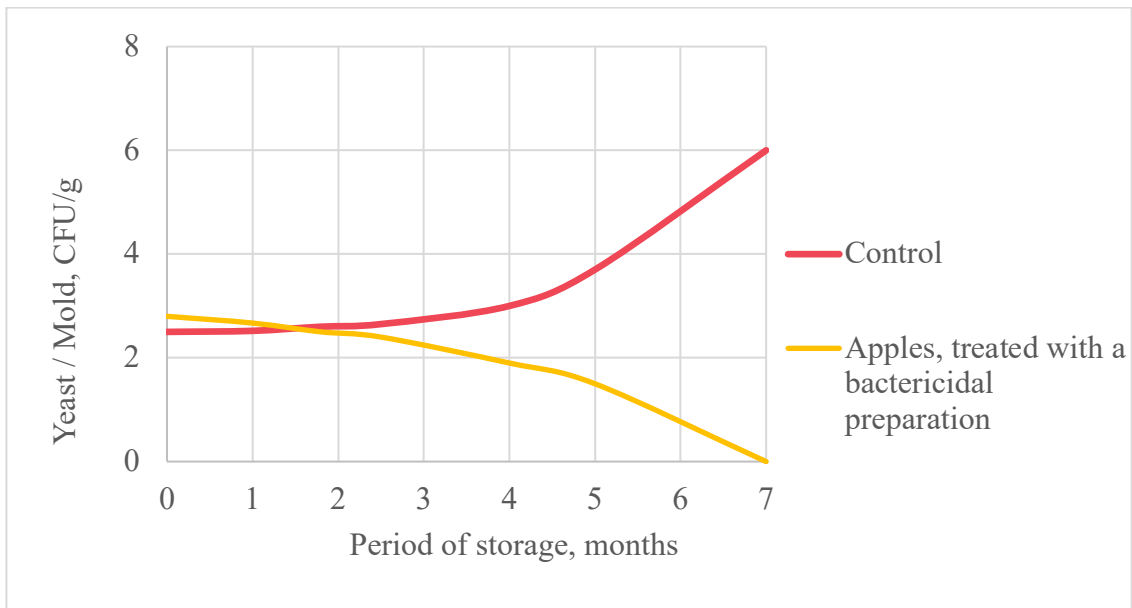


Figure 4 The effect of the apple storage method on microbiological indicators.

The tasting evaluation of apple fruits after 7 months of storage shows that the experimental samples exceeded the control ones in taste, presentation, and dense consistency (turgor of tissues) and no signs of wilting.

Thus, the results show that during long-term storage of apple fruits, there was a pronounced positive effect for fruits treated with a solution of the *Phytopsporin-M* biological preparation, which allowed to increase the yield of high-quality commercial products by 8 – 9%, which positively affected the economic indicators of production.

CONCLUSION

A low-cost technology of non-generator storage of apple fruits in an isolated "closed loop" has been developed. It allows you to implement the controlled atmosphere (CA) technology in a typical environment (7 – 8% CO₂) and (13 – 14% O₂) and a subnormal environment (3% O₂ and 5% CO₂) without using a gas generator. The technology of apple fruit storage with pretreatment of raw materials, containers, and internal storage surfaces with a solution of the biological preparation *Phytopsporin-M* are scientifically substantiated. The developed technology made it possible to reduce the moisture loss of the studied raw materials significantly, losses from microbial spoilage, and increase the yield of high-quality commercial products by 8 – 13%, which positively affected the economic indicators of production. Thus, our research aimed at extending the shelf life and reducing the loss of raw materials will provide an opportunity to increase the output of valuable canned fruit, improve the assortment, reduce the cost and generally increase the efficiency of their production.

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