CHAPTER 3

The effect of protective composition on the quality of sweet cherry during storage

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Abstract

The effect of an exogenous protective composition based on lactic and acetic acids on the preservation of sweet cherry fruits during refrigerated storage was studied. The physiological and biochemical mechanisms of action of this composition were established. The object of the study was sweet cherry fruits (*Prunus avium* L.) of nine pomological varieties: Rubinova Rannia, Valerii Chkalov, Kazka, Talisman, Dilema, Melitopolska chorna, Karina, Regina, Krupnoplidna. Samples were selected in the phase of consumer ripeness, taking into account varietal uniformity, degree of ripeness and fruit diameter. Before storage, the fruits were precooled: by intensive air method, hydrocooling in water and a combined method, which involved hydrocooling in aqueous solutions of organic acids with subsequent cooling with cold air. The cooled fruits were stored in a cold room at a temperature of 1.5 ± 0.5°C and a relative humidity of 93 ± 1%. The fruits were stored in cooling conditions for 40 days. The results suggest that the formation of the integral loss index is determined primarily by the method of pre-cooling the fruits, while varietal characteristics modify, but do not determine the nature of the post-harvest preservation of the fruits. The maximum yield of standard fruits was recorded in the experimental version of combined cooling with the use of an exogenous protective composition. It was found that cooling with a protective composition based on organic acids contributed to the reduction of the oxidative load in the tissues of sweet cherry fruits after 40 days of storage, which is confirmed by a lower level of malondialdehyde and a more balanced response of antioxidant enzymes.

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Taking into account the experimental data obtained, the practical significance of the proposed technology acquires a clearly expressed economic value. Under the conditions of selling 1 ton of chilled cherries, the revenue is 23 thousand UAH, while the value of the additional net profit obtained for the implementation of the proposed technology increases by almost 12,92 thousand UAH per 1 ton of product. The socio-economic effect of increasing the yield of standard products when stored using a combined method is 2918,2 UAH per 1 ton of fruit.

Keywords

Sweet cherry, cooling, storage, lactic acid, acetic acid, oxidative stress, malondialdehyde, antioxidant enzymes, microbiological diseases, physiological disorders, standard products, post-harvest losses.

3.1 Introduction

Sweet cherry (*Prunus avium* L.) belongs to the most valuable stone fruit crops, characterized by high consumer attractiveness in the fresh state, a limited storage life, and increased sensitivity to transportation and storage parameters. The global market of fresh sweet cherry exhibits stable demand, whereas international trade is concentrated in specific production regions and exhibits a pronounced seasonal pattern. This determines increased requirements for the stability of quality attributes and the predictability of product preservation within the cold chain system. Marketability of sweet cherry is determined not only by product mass but also by compliance with established standards regarding external appearance and textural characteristics [1]. Therefore, under cold chain transporting conditions, even a minor increase in the share of fruit exhibiting defects can significantly reduce the commercial value of the respective lot.

The scale of post-harvest losses also has a broader systemic dimension. According to estimates by the Food and Agriculture Organization of the United Nations, the global share of food loss at stages from harvest to retail amounts to approximately 13.3%, with fruit and vegetable products traditionally being among the most vulnerable categories due to high moisture content, intensive metabolism, and sensitivity to mechanical damage and microbial contamination [2]. Therefore, the reduction of post-harvest losses of fruits is considered one of the most effective approaches to increasing food system efficiency and reducing the resource burden on agri-food systems [3]. For sweet cherry, this issue is particularly acute: the crop belongs to highly perishable fruits, in which quality is determined by a delicate balance between physiological senescence, tissue water status, and the suppression of microflora growth.

The biological characteristics of sweet cherry significantly limit storage duration in the absence of specialized post-harvest regulatory technologies. Sweet cherry fruit possess a relatively delicate skin, high water content, and active post-harvest metabolism, which create preconditions for rapid senescence and texture loss. Simultaneously, during cold storage and transportation, the risk of microbiological diseases increases, representing one of the principal causes of marketability loss and reduction of the marketing period [4].

Contemporary studies on sweet cherry storage indicate that quality degradation in the post-harvest period is multifactorial and caused by: (i) physiological senescence accompanied by disruption of tissue structural integrity; (ii) development of oxidative stress, accumulation of malondialdehyde, and imbalance of antioxidant enzymes; (iii) microbiological contamination of the fruit surface followed by the development of microbial diseases under favorable conditions [5]. In addition, even under optimal temperature regimes, mechanical impacts during harvesting and sorting may cause localized tissue injuries, which intensify quality losses and increase fruit susceptibility to infection by pathogenic microorganisms [6]. In response to these challenges, post-harvest technologies in recent years have increasingly shifted from traditional approaches toward exogenous stability regulators with an improved safety profile. They combine antimicrobial effects, minimal risk of undesirable changes in sensory properties of product, and reduced technological and regulatory barriers. Recent research highlights growing interest in eco-friendly, safe postharvest solutions that effectively regulate physiological and biochemical processes in fruits, curb microbial diseases, preserve sensory qualities, and align with modern sustainability standards [7].

For sweet cherry, several approaches of exogenous regulation have proven their effectiveness, such as application of physical treatments and modified atmospheres, or natural bioactive compounds exhibiting antioxidant and antimicrobial properties [8]. However, the efficiency of such approaches is variable and depends on cultivar, maturity stage, pre-harvest conditions, and storage parameters, thereby necessitating the search for simple, reproducible, and technologically compatible solutions.

In this context, organic acids are considered a promising group of exogenous regulators, as they are capable of influencing the microbiological stability of the fruit surface through pH reduction, disruption of proton homeostasis in microbial cells, and inhibition of key metabolic processes. Recent review studies emphasize that organic acids are widely used as preservatives and acidity regulators and may demonstrate effectiveness against a broad spectrum of bacteria, yeasts, and fungi, being active under different temperature regimes. From the standpoint of post-harvest fruit

physiology, acid treatments also have the potential to indirectly affect the intensity of oxidative processes in tissues by reducing microbially induced damage and stabilizing surface barriers, which collectively may contribute to texture preservation and a reduction in the proportion of defects during storage [9].

Therefore, the relevance of studies devoted to investigating the effect of an exogenous protective composition based on lactic acid and acetic acid on the quality parameters of sweet cherry fruit during storage is determined by a number of interrelated factors: (i) the high commercial sensitivity of the crop to even moderate decreases in marketability; (ii) the dominant role of microbiological diseases and physiological senescence in the formation of post-harvest losses; (iii) the need for technologically simple, reproducible, and clean-label compatible solutions; and (iv) the necessity to move from fragmented quality indicators toward an integrated assessment of lot quality, in which the key criterion is the proportion of standard products after prolonged storage.

This conceptual framework – linking the regulation of microbiological stability and oxidative processes with the ultimate indicator of commercial quality – constitutes the scientific basis and determines the practical significance of the present study.

3.2 Theoretical foundations for the application of organic acids in post-harvest treatment

Lactic and acetic acids are low-molecular organic acids, the antimicrobial activity of which is due to a combination of physicochemical and biochemical mechanisms of action on the cells of microorganisms. Compared with other bactericidal and bacteriostatic substances, the mechanism of action of organic acids does not involve a direct effect on the process of protein denaturation and does not change the cellular structures of microorganisms. Organic acids in an undissociated form penetrate semipermeable membranes and dissociate. Protons formed as a result of dissociation reduce intracellular pH, and thereby disrupt the acid-base and energy balance of microbial cells. This causes acid stress. Under such conditions, the microbial cell is forced to spend a large amount of energy not on growth and reproduction, but on overcoming the effects of acid stress. This leads to rapid depletion of energy reserves and slowing down its growth [10].

Anions formed as a result of dissociation accumulate inside the cell and interfere with metabolic processes. They block the work of enzymes, disrupt logistical chains, the synthesis of proteins, DNA and other important metabolites. It should be noted that the mechanism of destructive action of anions is not universal and differs for

different types of acids. Thus, anions of acetic acid – acetates – mainly disrupt the energy chains of the microbial cell, which are connected with acetyl – CoA. In turn, anions of lactic acid – lactates – unbalance the redox system of microbial cells and inhibit the processes of their respiration [11].

When choosing organic acids for the formation of protective antimicrobial compositions for the treatment of fruit products, it is also important to take into account their physicochemical properties, as well as the conditions of use, in particular concentrations, temperature indicators, quantitative and species composition of microflora. Particular attention should be paid to the ratio of dissociated and undissociated forms of acids, which is determined by the pH indicator. The lower the pH value, the more undissociated forms in the solution and the more pronounced the antimicrobial effect [9].

The intensity of these mechanisms is determined by the physicochemical characteristics of the acids themselves and by environmental conditions. The effectiveness of lactic and acetic acids depends on their acid dissociation constant (pK_a), concentration, temperature parameters, and the composition of the surface microbiota of the fruit. Under post-harvest treatment conditions, particular importance is linked to the ratio between dissociated and undissociated forms, since the latter determines membrane permeability and the expression of bacteriostatic or fungistatic effects [12].

However, the mechanism of action of organic acids during post-harvest processing is not limited to the level of the microbial cell, but extends to the microenvironment of the fruit surface and its apoplastic space. The result of processing with protective compositions containing organic acids is an increase in the acidity of the cuticular layer and the surface layer of the epidermis, which inhibits contamination and further development of pathogenic microflora on the surface of storage objects [13]. The increase in the level of active acidity on the surface of fruit products affects not only the activity of microorganisms, but also inhibits the activity of enzymes. As a result, the rate of tissue destruction during storage decreases [14, 15]. Thus, post-harvest treatment with organic acids forms an acid barrier on the surface of storage objects, which reduces the risk of their microbiological spoilage during further storage.

Reducing the level of microbial contamination and the intensity of the development of microorganisms during storage has a positive effect on endogenous metabolic processes that occur during the storage of fruit products. The process of storing fruit raw materials is accompanied by the development of oxidative stress, the consequence of which is the accumulation of reactive oxygen species (ROS). First of all, these are hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radical (•OH). Intensive formation of reactive oxygen species induces peroxidation of cell membrane lipids, loss of ionic balance and accelerated tissue destruction [16].

Damage to plant tissues due to microbial contamination activates defense mechanisms, depletes them more quickly, and stimulates the development of oxidative stress [17]. On the other hand, reducing the level of contamination and the intensity of the development of pathogenic microflora when treating the surface of fruits with solutions of organic acids does not deplete the antioxidant system, reduces the level of formation of reactive oxygen species and inhibits the development of oxidative stress [18].

Therefore, the mechanism of action of organic acids in the composition of protective compositions for post-harvest processing of fruit products is characterized by both an external antimicrobial effect and indirect regulation of oxidative processes within tissues. This contributes to the extension of the shelf life of fruits with minimal loss of quality.

3.3 Conceptual framework and experimental modeling of the process

The scientific hypothesis of the study is based on the assumption that the application of an exogenous protective composition based on lactic and acetic acids, in complex with a combined cooling method, forms a controlled acidic barrier at the "surface – apoplast" interface of sweet cherry fruit. Such modification of the microenvironment is expected to reduce microbial load, limit pathogen penetration through micro-injuries, stabilize cellular membranes, and indirectly regulate the intensity of oxidative processes in the tissues. The implementation of these mechanisms is expected to reduce share of non-marketable fruit and increase the yield of standard products during prolonged cold storage.

Within the framework of this hypothesis, several working assumptions were formulated. It was proposed that the effectiveness of the acid composition may depend on cultivar-specific characteristics and the morphological structure of the fruit skin; that combined cooling would promote a more uniform temperature gradient within the tissues; that a reduction in microbial load would correlate with decreased lipid peroxidation levels; and that changes in apoplastic pH could serve as an early indicator of the depth of exogenous treatment effects and the stability of the cell wall structure.

The aim of the study was to provide experimental substantiation of the effect of an exogenous protective composition based on lactic and acetic acids on the storability of sweet cherry fruit under cold storage conditions and to elucidate the physiological and biochemical mechanisms underlying its action.

To achieve this aim, the following objectives were addressed:

– determination of the magnitude of microbiological and physiological losses during 40 days of storage;

– assessment of changes in natural weight loss and in the proportion of standard (marketable) products;

– calculation of the integral loss index and the share of standard products;

– investigation of the activity of antioxidant enzymes in fruit tissues;

– quantification of the intensity of lipid peroxidation based on malondialdehyde (MDA) content;

– analysis of the dynamics of apoplastic pH as an indicator of the acid–base status of the extracellular space;

– establishment of the relationships among microbiological, physiological, and biochemical quality indicators.

The object of the study was sweet cherry (*Prunus avium* L.) fruit of several varieties during the post-harvest period under cold storage conditions.

The subject of the study comprised changes in quality indicators, microbiological stability, and the redox status of fruit tissues under the influence of an exogenous protective composition based on organic acids within a combined cooling system.

At the experimental stage, the objects of analysis were fruits of nine sweet cherry cultivars: Rubinova Rannia, Valerii Chkalov, Kazka, Talisman, Dilema, Melitopolska chorna, Karina, Regina, and Krupnoplidna. Samples were collected at the stage of consumer maturity, taking into account cultivar uniformity, degree of ripeness, and fruit size (diameter).

After harvesting, the fruits were sorted to remove mechanically damaged and defective specimens and were bulk-packed into plastic crates (600 × 400 × 116 mm) with a net weight of 10 kg. Subsequent operations were carried out according to the developed experimental design, which included three pre-cooling treatments:

1. Control 1 (C1) – intensive air cooling. Cooling was performed using a forced stream of cold air at a velocity of 3.0 m/s with an air exchange rate of 90 volumes per hour. The chamber temperature was maintained at 0 ± 1°C with a relative humidity of 90 ± 1%.

2. Control 2 (C2) – hydrocooling. Cooling was carried out in a stationary pallet hydrocooler MAS-HC-2000-PAL-ST (capacity 2 t/h) using water at a temperature of 1.0 ± 0.5°C.

3. Experimental treatment (R) – combined cooling with an exogenous protective composition. The combined method consisted of two sequential stages. The first stage involved cooling in water at 1.0 ± 0.5°C supplemented with lactic acid (2.16%) and acetic acid (1.71%). The cooling duration was 10 ± 2 min, until the fruit core temperature reached 4 ± 1°C. The concentrations of organic acids were determined in preliminary studies as optimal for maintaining product quality and safety [19]. The second stage consisted of subsequent air cooling in a chamber with intensive air

circulation at a velocity of 3.0 m/s (90 volumes per hour) for 30 ± 2 min, until the fruit core temperature decreased to 2 ± 0.5°C. The operating temperature in the chamber was maintained at 0 ± 1°C with a relative humidity of 93 ± 1%. The total duration of the combined process was 40 ± 2 min.

After cooling, the fruits were placed in storage at a temperature of 1.5 ± 0.5°C and a relative humidity of 93 ± 1% in modernized cold chambers KH-48 equipped with an Eliwell EWDR 902 temperature control system and Eliwell EWHS 31 relative humidity sensors. The storage chambers were fitted with a battery-type cooling system. Temperature within the internal tissues was monitored using a digital thermometer TM-902CP with a type K thermocouple (measurement range −50 to +1300°C; resolution 0.1°C within −50 to 200°C). Each experimental treatment was performed in five replicates; one standard 10 kg crate constituted a single replicate.

To determine the effect of the exogenous protective composition on sweet cherry fruit quality during storage, a comprehensive evaluation of indicators was conducted, characterizing both the commercial suitability of the product and the biochemical mechanisms underlying its stability.

At the applied level, following parameters were analyzed: losses due to microbiological diseases, the proportion of fruit affected by physiological disorders, the magnitude of natural weight loss, and the share of standard (marketable) products after 40 days. Additionally, an integral index of reduction in commercial lot yield was calculated as a generalized indicator of product storability under cold storage conditions. Assessments were carried out in accordance with current regulatory methodologies, with changes monitored throughout the entire storage period.

Fruit exhibiting signs of microbiological infection and physiological disorders were recorded by complete inspection of each lot after every storage stage. The proportion of defective fruit was expressed as a percentage of the total number or mass of the sample according to the following formula

$$X = \frac{m_d}{m_0} \cdot 10, \tag{3.1}$$

where m_d – the mass of defective fruit, kg; m_0 – the initial mass of the specimen, kg.

Natural weight loss was determined by a gravimetric method through periodic weighing of crates containing fruit using analytical scales with an accuracy of 0.01 kg. Weight loss (%) was calculated as the ratio of the difference between the initial and current mass to the initial sample mass.

The proportion of standard (marketable) products was established in accordance with the requirements of current regulatory standards for the commercial quality of sweet cherry fruit.

The integral index of reduction in commercial lot yield was calculated as the total share of losses resulting from microbiological diseases, physiological disorders, and natural weight loss, allowing a comprehensive assessment of treatment effectiveness over 40 days of storage.

To provide a scientific substantiation of the mechanism of action of organic acids, biochemical indicators reflecting the state of oxidative homeostasis in fruit tissues were included in the evaluation system. In particular, the activity of key antioxidant enzymes – superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) – involved in ROS detoxification and regulation of cellular redox status was determined.

For enzymatic analyses, pulp samples (without stones) weighing 5–10 g were collected and immediately homogenized in chilled phosphate buffer (0.05–0.1 M, pH 7.0–7.8) at a ratio of 1:5 (w/v). The homogenate was centrifuged at 10,000–15,000 rpm for 15–20 min at 4°C. The supernatant was used to determine enzyme activity. All procedures were performed at low temperatures. Results were calculated on fresh weight (FW).

POD activity was determined by titration of residual hydrogen peroxide following pyrocatechol oxidation and expressed as μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$.

CAT activity was measured based on the rate of hydrogen peroxide (H_2O_2) decomposition by recording the decrease in absorbance at 240 nm. Activity was calculated from the change in H_2O_2 concentration over time and expressed as μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$.

APX activity was determined by monitoring the rate of ascorbate (AA) oxidation in the presence of hydrogen peroxide, recording the decrease in absorbance of the reaction mixture at 290 nm. Activity was calculated using the molar extinction coefficient for ascorbate (ε = 2.8 $mM^{-1} \cdot cm^{-1}$) and expressed as μmol AA $\cdot g^{-1} \cdot min^{-1}$.

SOD activity was determined based on its ability to inhibit the auto-oxidation of adrenaline in an alkaline medium, following the methodology in [20] with adaptation of the plant material preparation stage for analysis. For the assay, 0.5 g of finely ground plant tissue was homogenized in 5 mL of phosphate buffer (pH 10.65), using a mortar and pestle with added cooling medium (on ice) to prevent enzymatic degradation. The resulting homogenate was transferred to centrifuge tubes, after which 0.3 mL of chloroform and 0.6 mL of ethanol were added to precipitate impurities. The mixture was centrifuged at 8,000 rpm for 20 min. The supernatant was used for subsequent analysis, and the change in optical density was measured spectrophotometrically at 347 nm. SOD activity was expressed in arbitrary units as the percentage inhibition of adrenaline auto-oxidation relative to the control.

The intensity of lipid peroxidation was assessed by measuring MDA content using the thiobarbituric acid (TBA) reaction. After heating the reaction mixture and centrifugation, optical density was recorded at 532 nm with correction for non-specific absorption at 600 nm. MDA concentration was calculated using the molar extinction coefficient and expressed in nmol · g^{-1} of FW.

Additionally, apoplastic pH dynamics were monitored to assess changes in the acid-base status of the extracellular space and to determine the presence of a pH gradient at the "surface – apoplast" interface. Apoplastic pH was measured using a vacuum infiltration method with an isotonic solution, followed by centrifugation of the tissue to obtain apoplastic fluid. The pH of the extract was measured with a calibrated pH meter equipped with a microelectrode at 20 ± 1°C. These values were used as an indicator of the acid-base status of the extracellular space in fruit tissues.

All determinations were performed in five biological replicates. Statistical processing of experimental data was carried out using Microsoft Excel 365 software (Microsoft Corp., USA). The results were presented as the mean and standard deviation. To assess the influence of the cooling method, variety and their interaction, a two-factor analysis of variance (two-factor ANOVA) was used. Differences were considered statistically significant at $p \leq 0.05$. To characterize the share of the influence of individual factors, the η^2 indicator was additionally determined.

3.4 Effect of exogenous protective composition on the formation of sweet cherry fruit losses during storage

The effectiveness of postharvest treatments for sweet cherry fruits should be evaluated not only by individual quality indicators but primarily through a system of quantitative loss characteristics that develop throughout the storage period. The structure of total losses is multi-component and includes natural weight loss, determined by the intensity of transpiration and respiration; losses due to microbiological damage associated with the development of phytopathogenic microflora; and losses caused by physiological disorders resulting from disruption of water balance, membrane stability, and enzymatic equilibrium in the tissue. The combined effect of these factors ultimately determines the yield of standard (marketable) products and the economic efficiency of the storage technology.

Stone fruits, including sweet cherries, are characterized by high respiration rates, thin cuticles, and increased sensitivity to mechanical damage, creating conditions for rapid development of both dehydration and microbiological contamination. Over a 40-day storage period, even minor changes in microbial load or tissue

water-retention capacity can lead to nonlinear increases in the proportion of non-marketable fruit. Therefore, quantifying each loss component is essential for justifying the applied treatment methods and comparing their effectiveness across different cultivars.

Given the multifactorial nature of postharvest degradation processes, there is a need for a unified criterion that integrates diverse types of losses into a single quantitative measure. In this study, the chosen criterion is the integral loss index, which reflects the total reduction in commercial yield as a result of the three main components.

The integral index of commercial yield reduction of the lot (I_{Σ}, %) was calculated as the sum of three loss components formed during fruit storage: losses due to microbiological damage, losses from physiological disorders, and natural weight loss.

The proportion of losses due to microbiological damage (L_{mic}, %) was determined as follows: after sorting the lot, fruits showing signs of microbial spoilage were selected. The loss fraction was calculated based on mass

$$L_{mic} = \frac{m_{mic}}{m_0} \cdot 10, \tag{3.2}$$

where m_{mic} – the mass of fruit with microbiological damages, kg; m_0 – the initial mass of fruit lot, kg.

The proportion of losses due to physiological disorders (L_{phys}, %) was determined as follows: fruits exhibiting pronounced physiological defects that rendered the fruit non-marketable without signs of rot (such as softening, wilting, pedicel darkening, or subcutaneous spotting) were selected. The loss fraction was calculated using the formula

$$L_{phys} = \frac{m_{phys}}{m_0} \cdot 100, \tag{3.3}$$

where m_{phys} – the mass of mass of fruits with physiological disorders, kg.

It should be noted that each fruit was counted in only one defect category (based on the dominant symptom) to avoid double counting.

Natural weight loss (L_{nw}, %) was calculated as the relative decrease in lot mass due to transpiration and respiration

$$L_{nw} = \frac{m_0 - m_t}{m_0} \cdot 100, \tag{3.4}$$

where m_t – the batch mass at the time of evaluation (prior to sorting), kg.

The integral index of commercial yield reduction was calculated as the sum of the above components

$$I_{\Sigma} = L_{mic} + L_{phys} + L_{nw}. \tag{3.5}$$

Value I_{Σ} was presented in % and used as a comprehensive quantitative assessment of postharvest losses and the effectiveness of the applied treatments over the 40-day storage period.

The yield of standard products (Y_{std}, %) was calculated using the formula

$$Y_{std} = 100 - I_{\Sigma}. \tag{3.6}$$

The results of the experimental study and the calculated losses are presented in **Table 3.1**.

The results indicate a pronounced effect of the pre-cooling method on both the level and structure of postharvest losses in sweet cherry fruits, regardless of cultivar. After 40 days of cold storage, the integral loss index in the air-cooling variant (C1) ranged from 12.426 to 13.597%, reflecting a typical decline in product quality under intensive air cooling without additional stabilization measures. In this variant, natural weight loss predominated (5.2–5.4 %), while losses due to microbiological damage and physiological disorders accounted for 3.8–4.8% and 3.4–3.8%, respectively. These findings highlight the dominance of transpiration-driven water deficit under conventional air cooling.

In the hydrocooling variant without acids (C2), the integral loss index increased to 13.836–15.470%, representing the highest values among the tested methods. Although weight losses were minimal (1.705–1.925%), the microbiological component increased substantially, reaching 7.684–8.913%, nearly double that of C1, while physiological disorder-related losses ranged from 4.025–4.841%. Surface wetting during hydrocooling reduced transpiration losses but created favorable conditions for microbial proliferation, resulting in a predominance of microbiological damage in the total loss structure.

The lowest integral losses were observed in the experimental variant (R), which combined lactic and acetic acids with a two-step cooling process that included the removal of residual surface moisture. Total losses were only 3.347–3.865%, 3.5–4.5 times lower than the control treatments. Microbiological losses decreased to 0.735–1.033%, physiological disorder-related losses ranged from 0.945–1.216%, and weight losses were 1.504–1.755%. The sharp reduction in the microbiological component (8–9 times lower than C2) confirms the efficacy of organic acids as

exogenous microbistatic agents. Concurrent air cooling effectively removed residual surface moisture, further limiting pathogen proliferation.

Table 3.1 Sweet cherry fruit losses after 40 days of storage

Cultivar	Treat-ment	Weight loss, %	Losses due to microbiological diseases, %	Losses due to physiological disorders, %	Integral loss index, %
Rubinova Rannia	C1	5.429 ± 0.249	4.285 ± 0.058	3.366 ± 0.154	13.080 ± 0.241
	C2	1.925 ± 0.045	8.913 ± 0.271	4.632 ± 0.193	15.470 ± 0.248
	R	1.755 ± 0.021	0.950 ± 0.017	1.071 ± 0.052	3.776 ± 0.041
Valerii Chkalov	C1	5.215 ± 0.226	3.822 ± 0.180	3.389 ± 0.097	12.426 ± 0.308
	C2	1.839 ± 0.060	8.401 ± 0.062	4.841 ± 0.039	15.081 ± 0.096
	R	1.739 ± 0.005	0.806 ± 0.003	1.068 ± 0.013	3.613 ± 0.009
Kazka	C1	5.412 ± 0.011	4.407 ± 0.072	3.605 ± 0.005	13.424 ± 0.065
	C2	1.876 ± 0.022	8.712 ± 0.101	4.619 ± 0.022	15.207 ± 0.076
	R	1.742 ± 0.009	0.999 ± 0.017	1.124 ± 0.047	3.865 ± 0.047
Talisman	C1	5.364 ± 0.089	4.106 ± 0.048	3.790 ± 0.098	13.260 ± 0.200
	C2	1.711 ± 0.013	7.732 ± 0.326	4.393 ± 0.073	13.836 ± 0.313
	R	1.511 ± 0.020	0.735 ± 0.009	1.101 ± 0.059	3.347 ± 0.069
Dilema	C1	5.445 ± 0.067	4.563 ± 0.220	3.442 ± 0.021	13.450 ± 0.245
	C2	1.721 ± 0.008	8.557 ± 0.044	4.032 ± 0.085	14.310 ± 0.089
	R	1.622 ± 0.024	1.033 ± 0.040	0.954 ± 0.037	3.609 ± 0.066
Melitopol-ska chorna	C1	5.427 ± 0.119	4.247 ± 0.196	3.617 ± 0.083	13.291 ± 0.240
	C2	1.705 ± 0.018	7.684 ± 0.073	4.539 ± 0.055	13.928 ± 0.090
	R	1.504 ± 0.056	0.909 ± 0.011	0.945 ± 0.011	3.358 ± 0.057
Karina	C1	5.225 ± 0.153	4.359 ± 0.226	3.566 ± 0.223	13.150 ± 0.185
	C2	1.811 ± 0.037	8.872 ± 0.062	4.025 ± 0.097	14.708 ± 0.109
	R	1.703 ± 0.081	0.918 ± 0.016	1.216 ± 0.063	3.837 ± 0.055
Regina	C1	5.387 ± 0.258	4.762 ± 0.140	3.448 ± 0.014	13.597 ± 0.316
	C2	1.823 ± 0.175	8.312 ± 0.133	4.444 ± 0.071	14.579 ± 0.212
	R	1.698 ± 0.082	0.961 ± 0.014	1.084 ± 0.072	3.743 ± 0.140
Krupno-plidna	C1	5.223 ± 0.061	4.274 ± 0.165	3.791 ± 0.193	13.288 ± 0.272
	C2	1.797 ± 0.141	8.057 ± 0.131	4.401 ± 0.041	14.255 ± 0.210
	R	1.689 ± 0.055	0.893 ± 0.038	1.092 ± 0.072	3.674 ± 0.089

Cultivar differences were moderate and did not alter the overall pattern of the technological factor's effect. The lowest integral losses in the experimental variant were observed for the Talisman and Melitopolska chorna cultivars, which may be associated with the morphological features of the skin and tissue density. At the same time, the effect of the cooling method substantially exceeded inter-cultivar variation, indicating the decisive role of technological treatment in determining postharvest stability.

A two-way analysis of variance of the integral loss index after 40 days of storage (**Table 3.2**) revealed a statistically significant influence of the cooling method ($F(2,108) = 48{,}517.07$; $p < 0.001$), cultivar ($F(8,108) = 25.80$; $p < 0.001$), and their interaction ($F(16,108) = 23.06$; $p < 0.001$). The total sum of squares was $SS_t = 3{,}223.8861$ ($df = 134$), with the main contribution to variation arising from the cooling method factor ($SS_A = 3{,}201.3415$), while the contributions of cultivar ($SS_B = 6.8100$) and the interaction ($SS_{AB} = 12.1715$) were considerably smaller. The mean square error was $MSE = 0.0330$ ($df = 108$).

The effect size confirms the dominance of the technological factor: η^2 for the cooling method was 0.9930, indicating a decisive influence of the applied technology on loss formation. The contribution of the cultivar factor was considerably lower ($\eta^2 = 0.00211$), yet statistically significant, while the significant interaction between factors reflects the cultivar-specific nature of fruit responses to different cooling methods.

These results confirm that the formation of the integral loss index is primarily determined by the technological regime, whereas cultivar-specific traits modulate – but do not dictate – the postharvest stability of the fruits.

The dominant role of the cooling method, as established by the two-way ANOVA, is also clearly reflected in the structure of the standard product yield at the end of the storage period (**Fig. 3.1**).

Table 3.2 Two-way ANOVA results

Source of variation	*SS*	*df*	*MS*	*F*	*p*-value	F_{crit} (0.05)	η^2
Cooling method (A)	3201,342	2	1600,671	48517,07	$3.05 \cdot 10^{-160}$	3.0804	0.9930
Cultivar (B)	6.810	8	0.851	25.80	$7.43 \cdot 10^{-22}$	2.0252	0.00211
A × B	12.172	16	0.761	23.06	$1.10 \cdot 10^{-27}$	1.7380	0.00378
Residual	3.563	108	0.033	–	–	–	0.00111
Total	3223,886	134	–	–	–	–	1.0000

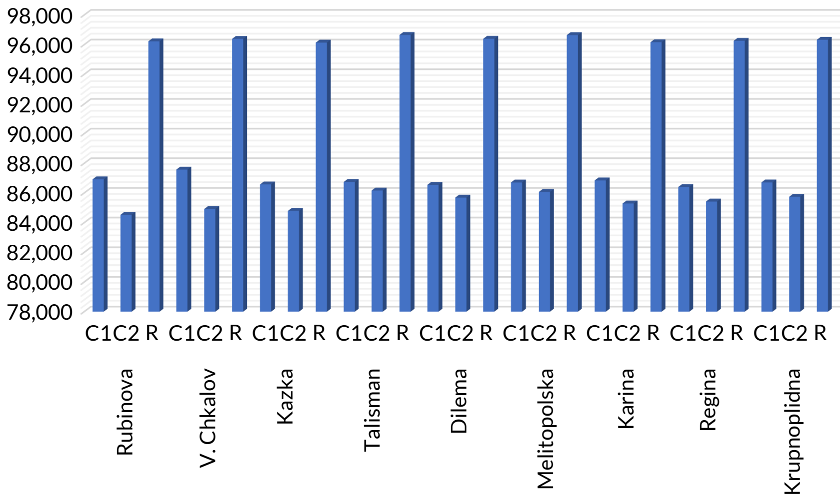

Fig. 3.1 Yield of standard sweet cherry fruits after 40 days of storage, %

The highest yield of standard fruits was observed in the experimental variant (R), which employed combined cooling with the use of an exogenous protective composition. For all cultivars studied, the yield exceeded 96%, with several cultivars approaching 97%.

Cultivar-specific differences in the absolute values of standard product yield persisted; however, their amplitude was substantially smaller compared to the effect of the cooling method.

These results demonstrate that the application of an exogenous acid composition within a combined cooling scheme ensures a consistently high yield of standard products regardless of cultivar, which is critically important for producing a predictable commercial batch during prolonged storage of sweet cherries.

3.5 Mechanisms of oxidative homeostasis regulation in sweet cherry fruits under the influence of the exogenous protective composition after 40 days of storage

Postharvest cooling of sweet cherry fruits is a critical stage that determines the subsequent course of metabolic processes during storage. The initial method of heat removal establishes the physiological response of the tissues, which is reflected

in the patterns of redox regulation, the intensity of ROS formation, and the stability of membrane structures throughout the storage period.

Sweet cherries are high-respiration fruits with increased sensitivity to oxidative stress. Disruption of the balance between ROS generation and antioxidant system activity leads to intensified lipid peroxidation, destabilization of cellular membranes, and accelerated senescence. MDA is considered an integrative marker of lipid peroxidation and an indicator of membrane structural integrity.

The enzymatic antioxidant system in fruits includes SOD, which catalyzes the dismutation of superoxide anions into hydrogen peroxide; CAT and APX, which further detoxify hydrogen peroxide; and POD, involved in regulating redox reactions within the cell wall and apoplast. The coordinated activity of these enzymes forms a cascade system that maintains redox homeostasis.

The results of MDA content determination in experimental sweet cherry cultivars under different pre-cooling methods after 40 days of storage are presented in **Table 3.3**.

Table 3.3 Malondialdehyde content in sweet cherry fruits after 40 days of storage depending on cultivar and cooling method, nmol · g^{-1} FW

Cultivar	C1	C2	R
Rubinova Rannia	6.34 ± 0.33	7.21 ± 0.37	4.02 ± 0.21
Valerii Chkalov	6.18 ± 0.29	7.05 ± 0.32	3.89 ± 0.18
Kazka	6.46 ± 0.31	7.38 ± 0.35	4.11 ± 0.20
Talisman	6.07 ± 0.26	6.86 ± 0.28	3.62 ± 0.16
Dilema	6.22 ± 0.28	7.12 ± 0.31	3.84 ± 0.17
Melitopolska chorna	6.10 ± 0.25	6.90 ± 0.29	3.55 ± 0.15
Karina	6.28 ± 0.30	7.26 ± 0.34	4.07 ± 0.19
Regina	6.49 ± 0.34	7.44 ± 0.38	4.16 ± 0.22
Krupnoplidna	6.16 ± 0.27	7.01 ± 0.30	3.92 ± 0.18

After 40 days of storage, MDA content showed a clear dependence on the postharvest cooling method. In all studied cultivars, the highest MDA levels were recorded in the hydrocooling variant (C2), ranging from 6.86 to 7.44 nmol · g^{-1} FW. On average, this variant exceeded the lipid peroxidation intensity under air cooling (C1) by approximately 14–16%.

In the intensive air cooling variant (C1), MDA values ranged from 6.07 to 6.49 nmol · g^{-1}, indicating a moderate level of lipid peroxidation after 40 days of storage. Despite the absence of direct contact with water, this method did not

provide sufficient stabilization of membrane structures, as TBA reactive compounds levels remained considerably higher compared to the combined cooling variant.

The lowest MDA values across all cultivars were observed under the combined cooling method with the application of the exogenous protective composition (R), with levels ranging from 3.55 to 4.16 nmol · g^{-1}. On average, lipid peroxidation intensity in this variant was 60% lower compared to C1 and 80% lower compared to C2. Thus, the use of organic acids in the protective composition reduced the accumulation of lipid oxidation products by approximately 1.6–1.8 times relative to the control cooling methods.

It is important to emphasize that the pattern of changes was consistent across all studied cultivars, indicating a systemic mechanism of action of the exogenous composition, independent of genotypic characteristics. Even for cultivars with relatively lower MDA levels in the controls, the combined method reduced lipid peroxidation intensity by at least 40%.

The obtained results are consistent with the integral loss indicators, which is confirmed by a strong positive correlation between MDA content and total product losses ($r = 0.95$; $p < 0.001$). The coefficient of determination ($R^2 = 0.90$) indicates that about 90% of the variation in postharvest losses is explained by the intensity of membrane lipid peroxidation.

The observed differences in MDA levels reflect the integrative outcome of oxidative processes in the tissues; however, to elucidate the mechanisms underlying this effect, it is necessary to analyze the status of the enzymatic antioxidant defense system (**Table 3.4**).

After 40 days of storage, the activity of antioxidant enzymes in sweet cherry fruits strongly depended on the postharvest cooling method. In all studied cultivars, coordinated changes in SOD, CAT, APX, and POD activities were observed, reflecting differences in oxidative load and the capacity for detoxification of reactive oxygen species.

SOD activity, which catalyzes the conversion of superoxide anion to hydrogen peroxide, remained within a relatively narrow range in the control variants, whereas in the variant with the exogenous protective composition (R), it was consistently higher. Compared to intensive air cooling (C1), SOD activity increased moderately in the hydrocooling variant (C2) by 3–5 arbitrary units depending on the cultivar. In variant D, the increase was more pronounced – on average 13–16 arbitrary units relative to C1 – indicating a higher capacity of tissues to neutralize ROS at the end of storage.

Changes in CAT and APX activities confirmed this trend. In C2, CAT and APX activities were slightly higher than in C1, whereas in R, the increases were more substantial: CAT activity was higher on average by 2.5–3.5 μmol H_2O_2 · g^{-1} · min^{-1},

and APX by 0.6–0.8 μmol AA · g^{-1} · min^{-1} compared to C1. This indicates more efficient hydrogen peroxide removal and a reduced likelihood of its participation in lipid peroxidation reactions.

Table 3.4 Activity of antioxidant enzymes in sweet cherry fruits after 40 days of storage

Cultivar	Treat-ment	SOD, % inhibition	CAT, μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$	APX, μmol $AA \cdot g^{-1} \cdot min^{-1}$	POD, μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$
Rubinova Rannia	C1	41.82 ± 2.11	7.63 ± 0.42	1.62 ± 0.09	43.82 ± 1.93
	C2	45.33 ± 2.32	8.02 ± 0.51	1.73 ± 0.10	22.44 ± 1.12
	R	56.74 ± 2.54	10.41 ± 0.60	2.28 ± 0.12	19.59 ± 0.92
Valerii Chkalov	C1	40.93 ± 1.89	7.38 ± 0.42	1.58 ± 0.08	45.26 ± 2.01
	C2	44.13 ± 2.22	7.83 ± 0.44	1.70 ± 0.09	25.66 ± 1.33
	R	55.41 ± 2.41	10.12 ± 0.56	2.22 ± 0.11	20.13 ± 1.08
Kazka	C1	42.61 ± 2.07	7.73 ± 0.55	1.66 ± 0.09	40.16 ± 1.82
	C2	46.05 ± 2.47	8.14 ± 0.46	1.78 ± 0.10	21.38 ± 0.93
	R	57.82 ± 2.63	10.61 ± 0.67	2.34 ± 0.12	18.18 ± 0.72
Talisman	C1	39.83 ± 1.84	7.16 ± 0.42	1.52 ± 0.08	38.27 ± 1.65
	C2	43.07 ± 2.18	7.52 ± 0.41	1.64 ± 0.09	17.48 ± 0.82
	R	53.96 ± 2.37	9.74 ± 0.52	2.12 ± 0.10	20.31 ± 0.94
Dilema	C1	41.27 ± 1.92	7.52 ± 0.44	1.60 ± 0.08	41.18 ± 1.74
	C2	44.63 ± 2.21	7.95 ± 0.53	1.72 ± 0.09	17.37 ± 0.82
	R	55.81 ± 2.47	10.27 ± 0.61	2.24 ± 0.11	14.15 ± 0.72
Melitopol-ska chorna	C1	40.13 ± 1.82	7.24 ± 0.44	1.54 ± 0.08	42.15 ± 1.62
	C2	43.45 ± 2.11	7.64 ± 0.42	1.66 ± 0.09	17.43 ± 0.85
	R	54.19 ± 2.32	9.84 ± 0.45	2.14 ± 0.10	20.13 ± 0.93
Karina	C1	41.6 ± 2.0	7.63 ± 0.55	1.61 ± 0.09	40.22 ± 1.82
	C2	45.1 ± 2.3	8.04 ± 0.57	1.74 ± 0.10	18.56 ± 0.87
	R	56.4 ± 2.5	10.35 ± 0.61	2.26 ± 0.12	15.32 ± 0.78
Regina	C1	42.93 ± 2.16	7.83 ± 0.52	1.67 ± 0.10	48.63 ± 2.17
	C2	46.43 ± 2.42	8.24 ± 0.57	1.80 ± 0.10	22.96 ± 1.17
	R	58.29 ± 2.74	10.73 ± 0.61	2.36 ± 0.13	20.25 ± 1.08
Krupno-plidna	C1	40.73 ± 1.94	7.32 ± 0.45	1.57 ± 0.08	40.13 ± 1.72
	C2	44.04 ± 2.26	7.68 ± 0.42	1.69 ± 0.09	16.25 ± 0.81
	R	55.13 ± 2.47	10.03 ± 0.64	2.20 ± 0.11	14.13 ± 0.74

The most pronounced differences were observed for POD. In C1, its values were the highest, ranging from 38 to 49 μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$ depending on the cultivar. In C2, POD activity decreased to 16–26 μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$, and in R to 14–22 μmol $H_2O_2 \cdot g^{-1} \cdot min^{-1}$. Thus, compared to intensive air cooling, hydrocooling and the combined method with the exogenous composition reduced POD activity 1.7–2.5-fold.

Considering POD localization in the cell wall and apoplast, these differences can be linked to varying intensities of oxidative processes in the extracellular space. High POD activity in C1 corresponds to dehydration stress and more intense oxidative reactions under active air circulation. The reduced POD activity in C2 and R indicates lower apoplastic oxidative stress.

Overall, the enzymatic profile after 40 days of storage shows that combined cooling with an exogenous protective composition is accompanied by increased SOD, CAT, and APX activities while simultaneously reducing POD. This enzyme activity pattern aligns with decreased lipid peroxidation and minimal postharvest fruit losses.

To further clarify the role of the extracellular environment in these differences, apoplastic pH of fruit tissues was additionally analyzed after 40 days of storage (**Table 3.5**).

Table 3.5 Apoplastic pH of sweet cherry fruits after 40 days of storage

Cultivar	C1	C2	R
Rubinova Rannia	4.18 ± 0.05	3.86 ± 0.04	3.52 ± 0.03
Valerii Chkalov	4.22 ± 0.06	3.90 ± 0.05	3.55 ± 0.04
Kazka	4.15 ± 0.04	3.82 ± 0.04	3.48 ± 0.03
Talisman	4.10 ± 0.05	3.78 ± 0.03	3.46 ± 0.03
Dilema	4.17 ± 0.05	3.84 ± 0.04	3.50 ± 0.03
Melitopolska chorna	4.12 ± 0.04	3.80 ± 0.04	3.47 ± 0.03
Karina	4.16 ± 0.05	3.83 ± 0.04	3.49 ± 0.03
Regina	4.24 ± 0.06	3.91 ± 0.05	3.56 ± 0.04
Krupnoplidna	4.14 ± 0.05	3.79 ± 0.04	3.45 ± 0.03

After 40 days of storage, the apoplastic pH in the variant with the exogenous protective composition was consistently 0.6–0.7 units lower compared to intensive air cooling. Intermediate values were observed in the hydrocooling variant. This pattern was consistent across all studied cultivars.

The decrease in extracellular pH corresponds with the reduced POD activity in variant R and indicates less pronounced oxidative processes in the cell wall and apoplast. A more acidic microenvironment may limit the intensity of peroxide-dependent reactions and create less favorable conditions for the development of microbiological damage, which correlates with the minimal integral losses of the product.

Thus, in addition to the increased activity of intracellular detoxifying enzymes (SOD, CAT, APX), the reduction of apoplastic pH in the variant with the exogenous composition acts as an additional mechanistic factor for maintaining sweet cherry fruit quality during prolonged storage.

3.6 Conceptual summary of biochemical mechanisms for maintaining sweet cherry fruit quality under the influence of an exogenous protective composition

The study demonstrated that the use of a combined postharvest cooling method with an exogenous protective composition significantly reduced oxidative stress in sweet cherry fruit tissues after 40 days of storage compared to the control variants of intensive air (C1) and hydrocooling (C2). This is evidenced by the lower content of MDA – a key marker of membrane lipid peroxidation – in variant R, whereas its accumulation was significantly higher in the C1 and C2 controls. The reduction of TBARS-products in the composition-treated variant indicates stabilization of membrane integrity and limitation of oxidative stress, which aligns with the well-established role of MDA as an integral indicator of lipid peroxidation [21].

Analysis of the enzymatic response of the antioxidant system revealed coordinated changes in the activity of SOD, CAT, APX, and POD depending on the cooling method. In variant R, the highest activities of SOD, CAT, and APX were recorded, indicating an enhanced capacity of the tissues to neutralize reactive oxygen species. As is well known, SOD catalyzes the dismutation of superoxide anion into H_2O_2, while CAT and APX ensure further detoxification of hydrogen peroxide, preventing its accumulation and the initiation of lipid peroxidation reactions. Similar patterns have been reported in postharvest physiology studies, where increased activity of antioxidant enzymes is associated with reduced MDA levels and improved preservation during cold storage [22].

Notably, POD activity was highest in variant C1, where oxidative stress was more pronounced, whereas in variants C2 and R, its level was substantially lower. Considering the POD localization in the apoplast and cell wall, these differences reflect

the varying intensity of extracellular oxidative processes. Additionally, in variant R, the apoplastic pH was lower compared to the controls, which could have modified the activity of cell wall-associated enzymes and limited the progression of peroxide-dependent reactions. It is known that apoplastic acidity influences the intensity of redox processes and the functioning of the peroxidase system, which is consistent with the obtained results [21].

The integration of biochemical and physiological indicators demonstrates a clear pattern: the control variants with intensive air and hydrocooling exhibited higher oxidative stress (elevated MDA and POD levels) and greater postharvest losses, whereas the combined variant with the protective composition ensured minimal accumulation of lipid peroxidation products with an optimized enzymatic response and the lowest product losses. This relationship between biochemical markers of oxidative stress and fruit quality indicators aligns with international studies, where activation of the antioxidant system is considered a key mechanism for prolonging storage life [23, 24].

Considering the obtained experimental and calculated data, the practical significance of the proposed technology acquires a clearly defined economic value. The application of the combined cooling method with an exogenous protective composition ensures the reduction of integral postharvest losses to a minimal level (around 3–4%), directly increasing the yield of standard products and the proportion of marketable fruits.

With marketing 1 ton of cooled cherry fruits, revenue amounts to 23,000 UAH (550 EUR), while the additional net profit from implementing the proposed technology increases by almost 12,920 UAH per ton of product (307–308 EUR). The socio-economic effect from increasing the yield of standard products during storage using the combined method amounts to 2,918.2 UAH per ton of fruits (69–70 EUR).

Thus, the biochemical stabilization of oxidative homeostasis through the use of the exogenous protective composition affects not only the improvement of the physiological state of the tissues but also produces a measurable economic outcome. The combined cooling technology enhances the profitability of cherry fruit storage and strengthens their competitiveness within extended logistical chains and market distribution.

Conflict of interest

The authors declare that there is no conflict of interest in relation to this paper.

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Data availability

Manuscript has no associated data.

Use of artificial intelligence statement

The authors used the AI assistant Perplexity (Grok 4.1, Perplexity AI) for translation and literature source selection. The authors bear full responsibility for the final manuscript. Generative AI tools are not credited and are not responsible for the final results.

Authors' contributions

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Tetiana Tymoshchuk: Writing – original draft, Writing – review and editing, Visualization, Formal analysis, Validation.

Olga Pyurko: Writing – original draft, Visualization, Formal analysis, Validation.

Sergii Basanets: Resources, Formal analysis, Visualization.

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