

THE ENZYMATIC ACTIVITY OF SOUR CHERRY FRUITS DURING STORAGE WHEN TREATED WITH A CARRAGEENAN SOLUTION

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Abstract: *The article is devoted to the effect of pretreatment with a solution of carrageenan on the enzymatic activity of cherry fruits in the period of storage. For the purpose of research between 2019-2020, the fruits of the Alpha and Pamiat of Artemenko cherry varieties of the same color were selected, sorted, washed and dried, covered with a solution that included carrageenan (1–2 g/100 ml of solution), glycerin (0.6 g/100 ml of solution) according to the options: without treatment (control) and treated with carrageenan solutions of 1% and 2% concentration. Cherry fruits were immersed in a pre-prepared solution, kept for 1-2 minutes, removed, allowed to drain and dried at the flow of air created artificially by a fan. Experimental and control samples of fruits were placed in boxes and stored at a temperature of $1.0\pm 0.5^{\circ}\text{C}$ and a relative humidity of $95.0\pm 1.0\%$. The most effective was the treatment of cherry fruits with a 2% solution of carrageenan, which made it possible to reduce the activity of antioxidant enzymes catalase by 38.5–35.7% and superoxidedismutase (SOD) by 9.5–11.1%. Between the antioxidant enzymes (CAT, SOD, APX) of cherry fruit, close correlations ($r = 0.86$, $r = 0.82$) were established and the regression equation was derived.*

Key words: *storage, cherry fruit, antioxidant enzymes, catalase, superoxidedismutase, ascorbatperoxidase, carrageenan.*

1. Introduction

Cherry fruits begin to deteriorate already at the stage of harvesting and storage, which causes significant product losses. Almost 25-45% of fruits are lost on the way of transportation and storage as a result of overripe, softening, weight loss,

disease development and microbiological spoilage [8], [10]. An important factor that prevents microbiological spoilage of fruits is their proper storage and packaging [29].

The existing plant protection products adversely affect the environment, since they use synthetic non-biodegradable

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packaging materials [5], [14]. The use of synthetic films, due to their insolubility, now causes significant environmental pollution [13], [26]. The solution to this issue is the use of edible biodegradable films and coatings, which consist of natural substances that are biodegradable, applied in a thin layer to the fruit and thus provide protection against moisture and function as a gas barrier.

According to EU Directive No. 95/2/EC of 1995 and EU Regulation 1333/2008, edible coatings consist of food ingredients, food additives, substances in direct contact with food, or food packaging. They are included in the edible part of the products and therefore must meet all the regulated requirements for the components contained in the food. Therefore, now there are a number of current innovations and developments to preserve the quality of fruits. These include the use of natural compounds, food coatings and films, active packaging, nanotechnology etc. [27], [30-32]. Most often, polysaccharides, chitosan and alginates are used as food coatings [6]. These are polymeric carbohydrates consisting of monosaccharides interconnected by glycosidic bonds.

These compounds are widely available in nature, are part of algae, plants, microorganisms and animals [17].

Since the end of the XX century, given the global trends in combating environmental pollution and the current problem of putting into operation innovative environmentally friendly materials, edible coatings are increasingly used to preserve the quality of various food products. They have several advantages over widely used synthetic polymer packaging. After application to

the product, edible coatings become an integral part and are consumed with it.

Carrageenan is a natural polysaccharide of Irish moss (*Chondrus Crisp*) and certain types of red algae that form gels at low concentrations in water [3] and are used to store fruits [16]. An increase in the duration of storage of fruits leads to a decrease in the content of nutrients, including enzymes in fruits (sweet cherries and jujubes) [15], [37]. Enzymes affect oxidative damage to the membrane, which is the result of aging of the fruit [18]. Enzymes, including ascorbateperoxidase, are involved in the transfer of hydrogen peroxide [25]. Catalase is one of the important enzymes that protects the cell from oxidative damage, by reducing the passage of oxidative processes [37].

Processing fruits before storage with chitosan polysaccharide, salicylic acid, reduces oxidative stress, improves fruiting and slows down the aging of fruits after storage. Chitosan coating delays fetal aging, which is associated with enzymatic and non-enzymatic antioxidant systems [1], [4], [23].

The non-enzymatic antioxidant system of fruits, in particular cherries and cherries, includes phenols and antioxidants. Enzymes such as catalase, peroxidase and superoxide dismutase (CAT, APX and SOD) are fundamental for oxygen absorption and prevention of cell oxidation [23]. The enzyme catalase exhibits an antioxidant effect and catalyzes the decomposition of hydrogen peroxide into water and oxygen, reducing the harmful effects caused by free radicals. The enzyme superoxidisedismutase plays an important role in protecting cells from cancer diseases [9].

The pre-processing of fruits before storage affects their enzymatic activity. The treatment with a solution of chitosan of peach fruit leads to a change in polyphenoloxidase, which first increases storage and then decreases it. Peroxidase is an antioxidant enzyme that catalytically decomposes hydrogen peroxide in lignin biosynthesis. The peroxide activity in fruits treated with chitosan is higher than in untreated fruits [12], [33].

Oxidative stress in plant cells involves the accumulation of free radicals, reactive oxygen forms (ROS), such as a superoxide radical. ROS is generated in plant cells, due to metabolism in reactions catalyzed by oxidase and lipoxygenase. As a result of β oxidation of fatty acids, they are constantly removed by the enzymatic and non-enzymatic systems. Consequently, the ROS content in plant cells depends on their producing systems and the removal mechanism. Non-enzymatic compounds include reduced forms of ascorbate, tocopherols, phenols, alkaloids, and enzymatic mechanisms – superoxide dismutase, catalase, peroxidase, ascorbatperoxidase. Despite the presence of these systems, oxidative damage occurs in plant cells due to the inefficient disposal of ROS under stressful conditions and is associated with aging during storage.

A low concentration of malonic dialdehyde may be associated with the synergistic effect of chitosan with salicylic acid. Chitosan enhances the activity of catalase in cherries. Oxidative stress and the accumulation of oxygen and hydrogen peroxide and the protection from it depend on the presence of antioxidant enzymes such as superoxidedismutase, catalase, ascorbatperoxidase, preventing its appearance [28], [34], [36].

2. Materials and Methods

2.1. General Information

For the experimental studies, between 2019-2020, the fruits of cherries of the Alfa and Pamiat of Artemenko varieties of the same color were selected, sorted, washed and dried. The prototypes were covered in a solution that included carrageenan (China, Fengchen group Co., Ltd), 1–2 g/100 ml of solution, glycerin (Poland, Bioagra-Oli S.A.) 0.6 g/100 ml of the solution according to the options: without treatment (control) and treated with carrageenan solutions of 1 and 2% concentration. To dissolve, the mixture was heated at 80°C stirring for 30 minutes and then cooled. The fruits of cherries were immersed in the prepared solution, kept for 1-2 minutes, removed, allowed to drain and dried by a flow of air created artificially by a fan. Experimental and control samples of fruits were placed in boxes and stored at a temperature of $1.0\pm 0.5^{\circ}$ C and a relative humidity of $95.0\pm 1.0\%$ [20]. The preparation and selection of samples for analysis was carried out according to DSTU ISO 874-2002 [7].

In the period of storage, the activity of the enzyme catalase and ascorbatperoxidase and superoxide-dismutase of cherry fruit was determined. The criterion for the end of the fruit storage period was a weight loss of no more than 6% [20]. The repetition of the experiment is threefold.

The *catalase*, *ascorbateperoxidase* and *superoxidedismutase* antioxidant enzymes' activity which was determined in pulp and peel tissue samples (4 g) was initially prepared by homogenizing fruit tissue (peel or pulp) in a pre-cooled 0.1 M potassium phosphate buffer (pH 7.0). The mixture was centrifuged at 12,000× g, at 4°C, for 20 minutes [24].

The *catalase* (CAT) activity was assayed according to Xing et al. [35] with modifications. The reaction system consisted of 0.5 mL enzyme extract of 2 mL sodium phosphate buffer (50 mM) and 0.5 mL H₂O₂ (40 mM). The decomposition of H₂O₂ was measured by the decline in absorbance at 240 nm using a spectrophotometer. The CAT activity was expressed as U mol/min.

The *ascorbateperoxidase* (APX) activity was assayed according to Nakona and Asada [22]. The reaction solution included 0.1 M phosphate buffer (pH 7.0), 5 mM ascorbate, 0.5 mM H₂O₂, and the enzyme extract. Indicators were recorded at 290 nm using a spectrophotometer (UVvis).

The *superoxidedismutase* (SOD) activity was analysed according to Misra and Fridovich [18]. The reaction mixture was 200 mg of fresh tissue which was homogenized in a 5 ml (100 mM) K-phosphate buffer (pH 7.7) containing ethylenediaminetetraacetic acid (EDTA) (0.1 mM), 0.1% triton X-100 and 2% polyvinyl pyrrolidone. Then the extract was filtered through muslin cloth and centrifuged at 22000 × g for 10 min at 4-8°C. Then, the reaction mixture was dialyzed against the cold extraction buffer for 4 h with a carbonate/bicarbonate buffer. The mixture in a total volume of 3 ml contained a 50 mM sodium carbonate/bicarbonate buffer (pH 9.7), 0.1

mM EDTA, 0.6 mM epinephrine and enzyme. The adrenochrome formation in the next 4 min was recorded at 475 nm in a spectrophotometer.

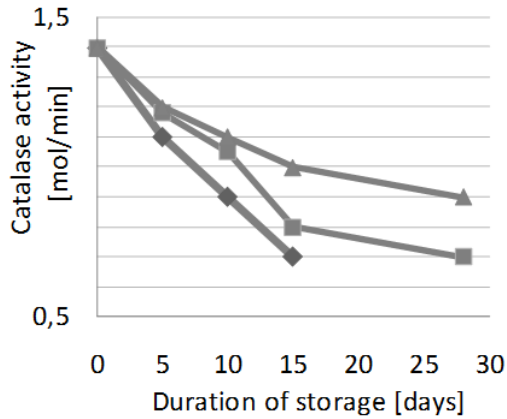
2.2. Statistical Analysis

Data were expressed as mean ± standard deviation; for mathematical data processing the value of $p < 0.05$ was regarded as statistically significant. Two-way analysis of variance (ANOVA) was used to determine the significance of differences. The statistical analyses were performed STATISTICA 6 to V. F. Moyseychenko [19] and the program "Excel 2000".

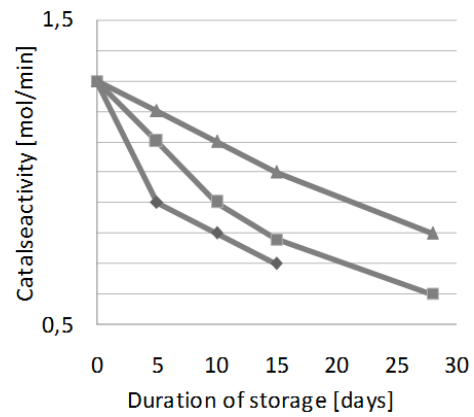
3. Results and Discussions

Thus, after 15 days storage of cherry fruit, the activity of the enzyme catalase decreased by 46-50% (Figure 1).

When treated with a 1 and 2% solution of carrageenan, the decrease in enzyme activity after 15 days was less – 40 and 42.9% and 23 and 28.6%, respectively, its lowest activity after 28 days of fruit storage was treated with a 2% solution of carrageenan – 35.7 and 38.5 %. Studies by Lin et al. [11] have established smaller losses of physicochemical components during storage of fruits treated by a solution of carrageenan. Due to the fact that a semi-permeable film is formed on the surface of the fruit, which is a barrier to the passage of oxygen and carbon dioxide in the fruit. The activity of ascorbatperoxidase during the entire storage period of control increased by 15.8-17.1% (Figure 2).



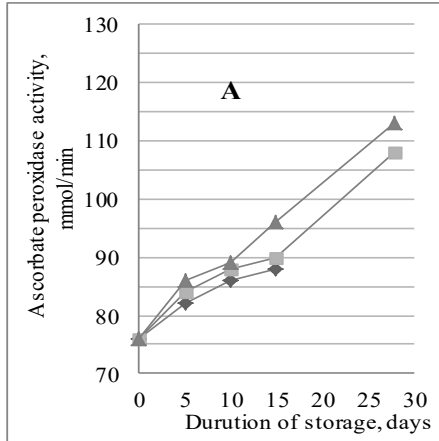
a.



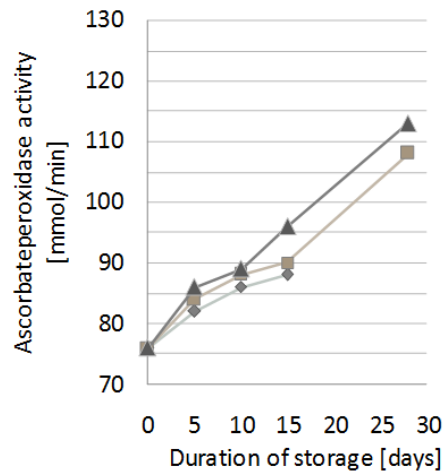
b.

- ◆ -- no treatment (control);
- -- 1% carrageenan solution;
- ▲ -- 2% carrageenan solution.

Fig. 1. Change in catalase activity in cherry fruits of Alfa (a.) and Memory of Artemenko (b.) varieties treated with carrageenan solution before storage ($LSD_{05}=0.03$)



a.



b.

- ◆ -- no treatment (control);
- -- 1% carrageenan solution;
- ▲ -- 2% carrageenan solution.

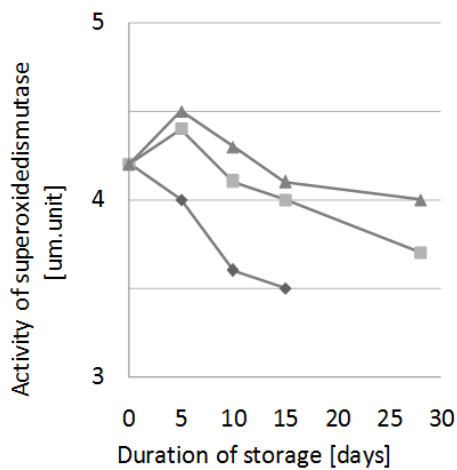
Fig. 2. Change in the activity of the enzyme ascorbatperoxidase in the fruits of cherries of varieties Alpha (a.) and Memory of Artemenko (b.), treated with a solution of carrageenan before storage ($LSD_{05} = 2.4$)

Significant increases in its activity were observed in cherry fruits treated with 1 and 2% carrageenan solution by 31.4-

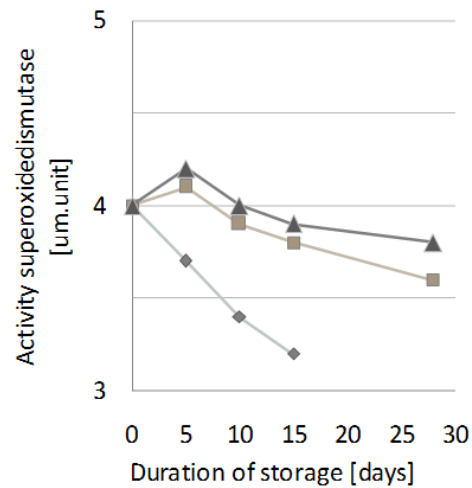
44.3% and 18.4-26.3%, respectively, after 15 days and by the end of storage by 42.1-58.6%. As can be seen from Figure 3, the

content of ascorbic acid during storage decreased, while the activity of ascorbateperoxidase (APX), increased in the entire storage period. Moreover, its

accumulation took place more actively for seven to eight days of storage and by the end of this period it was more stable.



a.



b.

- ◆ -- no treatment (control);
- -- 1% carrageenan solution;
- ▲ -- 2% carrageenan solution.

Fig. 3 Change in the enzyme superoxide dismutase in the fruits of cherries of varieties Alpha (a.) and Memory of Artemenko (b.), treated with a solution of carrageenan before storage ($LSD_{05} = 0.2$), 2019–2020

In contrast, in the treated cherries with a solution of carrageenan, the growth of APX took place within 10 to 15 days according to the Alpha variety, while the Pamiat of Artemenko variety was stable and increased rapidly by the end of storage. The antioxidant stress of the fruit during storage is prevented by the activity of the enzyme superoxide dismutase whose activity, during the storage of fruits in the control version decreased by 16.7-20.0% (Figure 4).

Slightly smaller changes were in cherry fruits treated with 1 and 2% carrageenan solution by 2.5-5.0% and 2.4-4.8%, respectively, and by the end of storage by 4.8-11.9%. Moreover, the lowest

activity (LSD) in the fruits of cherries treated with a 2% solution of carrageenan was 9.5-11.1%. Similar trends were established on strawberry fruits treated with sodium alginate, which had higher antioxidant activity and superoxide dismutase activity [2].

Strong correlations have been established between the activity of antioxidant enzymes and the rice regression equation has been derived 5.

Thus, a strong correlation was established between the activity of catalase and superoxide dismutase ($r = 0.86 \pm 0.001$), as well as between the activity of ascorbate peroxidase and superoxide dismutase ($r = 0.82 \pm 0.002$).

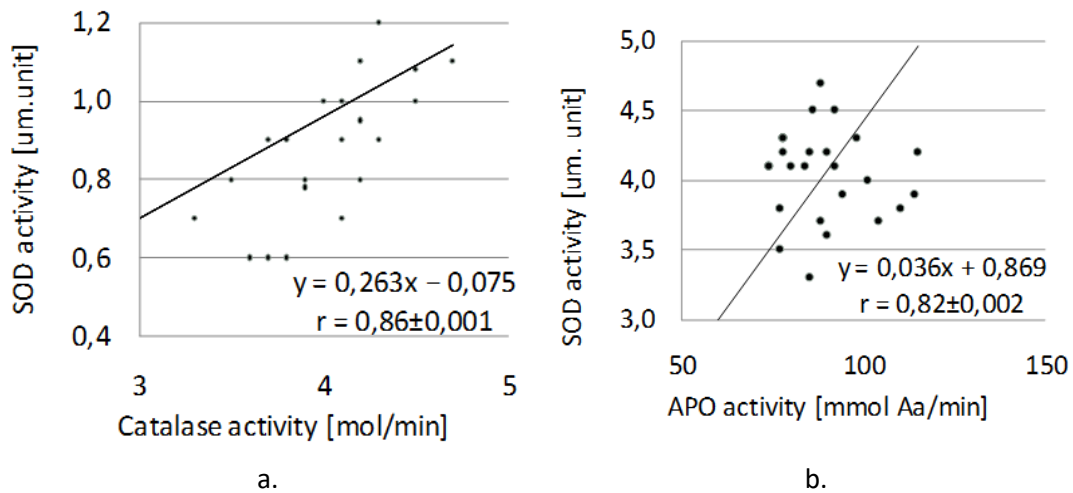


Fig. 4 Correlation dependences of the activity of SOD enzymes on the activity of CAT (a.), APX (b.)

4. Conclusions

So, the most effective was the treatment of cherry fruits with a 2% solution of carrageenan, which made it possible to reduce the activity of antioxidant enzymes catalase by 38.5-35.7% and superoxidedismutase (SOD) by 9.5-11.1%. The activity of ascorbateperoxidase (APX) was more stable throughout the storage period. Between the antioxidant enzymes (CAT, SOD, APX) of cherry fruit, close correlations ($r = 0.86$, $r = 0.82$) were established and the regression equation was derived.

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