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The effect of the preharvest treatment of sour cherry fruit (*Prunus cerasus* L.) with chitosan and mixture of chitosan and salicylic acid on fruit quality

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The effect of the preharvest treatment of sour cherry fruit (*Prunus cerasus* L.) with chitosan and mixture of chitosan and salicylic acid on fruit quality

Abstract: The effect of the pre-harvest treatment of sour cherry fruit with chitosan and salicylic acid on product quality as well as mass losses after storage is shown in the paper. The research was carried out with sour cherry cultivars 'Alfa' and 'Pamiat Artemenko' at the experimental pomology station named after L.P. Symyrenko of the institute of horticulture of NAAS. The pre-harvest treatment of sour cherry fruit with mixture of 1 %-chitosan with 100 mg l^{-1} of salicylic acid appeared to be efficient; this makes it possible to extend the storage period up to 30 days, to increase the output of marketable produce by 7.8–8.6 %, to have mass losses which do not exceed 3 %. The pre-storage treatment of sour cherry fruit with the solution of salicylic acid in combination with chitosan facilitates the decrease of the number of mesophylic aerobic and facultative anaerobic microorganisms by 5.2 times, that of yeast and mold – 6.3 times. Along with this, taste properties of the produce are maintained at the level of fresh fruit, also the product has an excellent tasting evaluation – 5 points.

Key words: product evaluation, mass loss, sour cherry fruit, chitosan, salicylic acid.

Učinek obravnavanja plodov višnje (*Prunus cerasus* L.) pred obiranjem s hitozanom in mešanico hitozana in salicilne kisline na njihovo kakovost

Izvleček: V prispevku je prikazan učinek tretiranja plodov višnje pred obiranjem s hitozanom in mešanico hitozana in salicilne kisline na njihovo kakovost in izgubo mase po spravilu. Raziskava je bila izvedena na sortah višnje 'Alfa' in 'Pamiat Artemenko' na poskusni postaji za sadjarstvo imenovani po L.P. Symyrenku, inštituta za hortikulturo NAAS. Obravnavanje plodov višnje pred obiranjem z mešanico 1 %-hitozana z 100 mg l^{-1} salicilne kisline se je izkazalo za učinkovito, kar je omogočilo čas shrambe do 30 dni in povečalo prodajno vrednost pridelka za 7,8–8,6 % ter zmanjšalo izgubo mase pod 3 %. Obravnavanje plodov višnje pred shrambo z raztopino salicilne kisline v kombinaciji s hitozanom je omogočilo zmanjšanje števila mezofilnih aerobnih in fakultativno anaerobnih mikroorganizmov za 5,2 krat, kvasovk in plesni za 6,3 krat. Hkrati so lastnosti okusa ostale na isti ravni kot pri svežih plodovih saj je bila ocean okusa odlična in je znašala 5 točk.

Ključne besede: ovrednotenje pridelka, izguba mase, plodovi višnje, hitozan, salicilna kislina

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1 INTRODUCTION

Fruits with a short shelf life, including sour cherries, have considerable respiration intensity with a high level of transpiration, they are susceptible to physiological diseases; so they spoil and are lost at the first stages of harvesting on the way from producers to consumers.

Cultivar, harvesting time, handling, and packaging greatly affect the quality and shelf life of sweet and sour cherry. However, its high perishability after harvest is mainly due to its high respiration rate, physical-chemical changes (such as mass loss, softening, darkening, mechanical damage etc.), and the development of microorganisms spoilage. Among them, primarily diverse species of filamentous fungi cause relatively faster spoilage after harvesting. The most important spoilage fungi of sweet cherries are *Botrytis cinerea* Pers., *Penicillium expansum* Link., and *Monilinia* spp. Therefore, strict storage conditions must be applied, alone or in a combination with different postharvest strategies, to delay spoilage and maintain quality (Cabañas et al., 2023).

At present new storage technologies in combination with existing ones are worked out – the effect of a low temperature, radiation, the use of the substances with anti-microbial effect/ aloe vera coating, 1-metylcyclopropen (Jianglian & Shaoying, 2013; Wani et al., 2014). However, the application of these substances for the extension of storage duration of cherry fruit has not been studied thoroughly, and preparation 1-MCP is very cost consuming to be used.

For chitosan to be effective in use, it is combined with other substances: essential oils, salicylic and metylsalicylic acids, etc. (Jianglian & Shaoying, 2013; Pasquariello et al., 2015).

Chitosan is a linear polysaccharide obtained by deacetylation of chitin, which, after cellulose, is the most abundant natural biopolymer in nature (Pereira, 2018). It has great potential for active packaging due to its biocompatibility, antimicrobial activity, non-toxicity and physical properties. Chitosan is active against pathogens and microorganisms, including bacteria (Thabet, 2019).

The chitosan coating has been approved by the (US-FDA) as a GRAS substance and its use is safe for the consumer and the environment. The use of chitosan in horticulture, and especially on highly perishable products (including sour cherry fruit), is based on the following properties such as cost, availability, functional attributes, mechanical properties (flexibility, tensile strength), optical properties (brightness and opacity), barrier effect against gas flow, structural resistance to water and microorganisms, and sensitivity (Romanazzi et al., 2017).

Salicylic acid and acetylsalicylic acid are plant hormones which play the main role in a wide spectrum of physiological processes. The prior salicylate treatment of fruit prevents spoilage, reduces damages and improves appearance and their density (Razavi et al., 2018). Salicylic acid is a natural and safe phenolic compound, it has a high potential as to the control over losses after harvesting. The use of salicylic acid is efficient to prevent damage of peach, kiwi, sweet cherry, apricot, pomegranate, plum (Youzuo et al., 2015).

Besides, the prior treatment of fruit with salicylic acid helps extend a storage period and preserve their quality. Peach fruit, treated with salicylic acid, were stored at 1 °C for 28 days. The content of phenols, flavonoids, ascorbic acid was higher as compared with the untreated fruit (Razavi et al., 2018).

Thus, the postharvest treatment with salicylic acid can be a safe, ecological technique aimed at preserving fruit quality (Zapata, 2017).

According to the research done by A. A. Lo'ay, A. Mohamed, M. A. Taher (2018), salicylic acid used in combination with chitosan enhances the resistance to damage of guava fruit during 15 days at temperature 27 °C. The effect of chitosan and salicylic acid on the storage of grapefruit is shown in the research (Shi et al., 2019). The pretreatment prevented a green mold formation, inhibited the effect of enzymes and contributed to the firmness of apricot, grapefruit, sweet cherry (Ghaouth et al., 1997; Gimenez et al., 2014; Shi et al., 2019; Cui et al., 2020).

The treatment of citrus fruit with oligochitosan, salicylic acid and Pichia membranaefaciens (E.C. Hansen) E.C. Hansen contributed to a significantly lower morbidity and damages during storage (Pereira, 2017; Xoca-Orozco et al., 2018).

However, not enough research was conducted concerning the effect of the combined use of salicylic acid and chitosan for the pretreatment of stone fruit, in particular sour cherries, on their quality (Vasylyshyna, 2018; Vasylyshyna & Chernega, 2022; Vasylyshyna, 2023). To improve the quality of fruit-berry output, the fruit treatment with salicylic acid in combination with chitosan and other substances is widely used (Zheng et al., 2007; Youzuo et al., 2015).

The aim of the study was to investigate the effect of pretreatment with a solution of chitosan and salicylic acid on the marketability, organoleptic and microbiological parameters of sour cherry fruits during storage.

2 MATERIAL AND METHODS

2.1 PRE-AND POST-HARVEST TREATMENT

The research was conducted with sour cherry cultivars 'Alfa' and 'Pamiat Artemenko' at the experimental pomology station named after L.P. Symyrenko of the institute of horticulture of NAAS in the years of 2016–2019. For the trial, the day before harvesting, 15 trees of each cultivar were sprayed with the solution of salicylic acid (100 mg l^{-1}); 1% chitosan mixture with salicylic acid (100 mg l^{-1}). Untreated sour cherry fruit were taken as the control. A three-time replication of the trial was applied. The fruit of a certain cultivar and a certain treatment technique were harvested at a consumer stage from four different places in the crown of each tree; they were placed for storage in boxes № 5 (5 kg each) at temperature 1 ± 0.5 °C and relative humidity 95 ± 1 % in an industrial refrigeration chamber KH with a capacity of 10.8 cubic meters. The control fruits were stored for 15 days, and the experimental ones – for 30 days.

2.2 ANALYTICAL METHODS

To determine product quality, sour cherry fruit of the first grade were chosen after storage. As to their appearance, they had a shape and coloring, typical for a given sour cherry cultivar. Sour cherry fruit were similar by the degree of ripeness, no overripe ones were there. The number of fruit without peduncle and with scarred injuries was not higher than 4 %. Fruit were of the same size with an average diameter not less than 16 mm (15.51 ± 0.05 mm), red color, they did not have injuries and they met the requirements of fresh sour cherries. Technical conditions (GSTU 01.1-37-167:2004). Fruits with mechanical damage exceeding 3 % and browning (5 % and higher) were considered to be of technical defects.

2.3 MASS LOSSES

At the end of storage, natural mass losses were determined by means of weighing on the scales (TWE) with an accuracy of 0.01 g. Mass loss was expressed in percents as the difference of two weighings before and after storage. The criterion of the end of storage was mass loss which would not exceed 6 % (Naichenko, 2001).

The determination of the number of mesophylic aerobic and facultative anaerobic microorganisms (MAFAnM) was done with help of cup method according to food products. Methods for determining the number of mesophilic aerobic and facultative anaerobic microorganisms (DSTU 8446:2015). By determining the number of mesophilic aerobic and facultative anaerobic microorganisms by sowing into solid product nutrient media, incubating the cultures, counting all visible colonies that have grown. For the determination, the following was used: (MPA) with contents (in %) – meat water, peptone – 1 %, agar–agar – 2 %. Endo's Medium (for defining of coliform bacteria) with contents (g dm^{-3}) – peptone – 5.0; triptone – 5.0; lactose – 10.0.

2.4 ORGANOLEPTIC EVALUATION

Organoleptic evaluation of sour cherry fruit (by sensory analysis) as to overall, sweetness, color, consistence, scent, taste, appearance, the consumers evaluated by 5-point scale (Naichenko, 2001). Scale range (0 to 5) with expression of perception (poor 0– 2, fair–3, good–4, excellent–5).

2.5 STATISTIC ANALYSIS

All statistic analyses were made at probability level 5 % ($p \leq 0.05$) with help of STATISTICA 10 (factorial ANOVA). The data is presented as average values with standard deviations of three measurements.

3 RESULTS AND DISCUSSION

As the research results showed, pretreatment of sour cherry fruit with chitosan with salicylic acid facilitated the extension of the storage duration up to 30 days, as compared with 15 days in the control.

The output of marketable produce (Fig.1) of sour cherry fruit 'Alfa' and 'Pamiat Artemenko' was at the same level after 15 days of storage – 85.2 and 83.6 %, respectively. The maximal output of marketable produce was recorded for the fruit pretreated with 1 % chitosan with salicylic acid – by 7.8–8.6 % higher as compared with the control, absolute waste was 2.3 times (3.1–3.6 %).

Mass losses of fruit result from respiration and moisture transpiration. During storage of sour cherry fruit 'Alfa' and 'Pamiat Artmenko', mass losses in the control were at levels 5.4–5.7 % (Fig. 2). The pretreatment of sour cherry fruit with salicylic acid resulted in the decrease of mass losses up to 3.4–3.5 %, and the treatment with chitosan in combination with salicylic acid – up to 2.7–3.0 % during 30-day storage; it occurred due to semi-permeable coating formed on fruit surface which prevented moisture losses and reduced respiration intensity; this was proved by researchers Z.Youzuo, Z. Meiling.,Y. Huqing (2015).

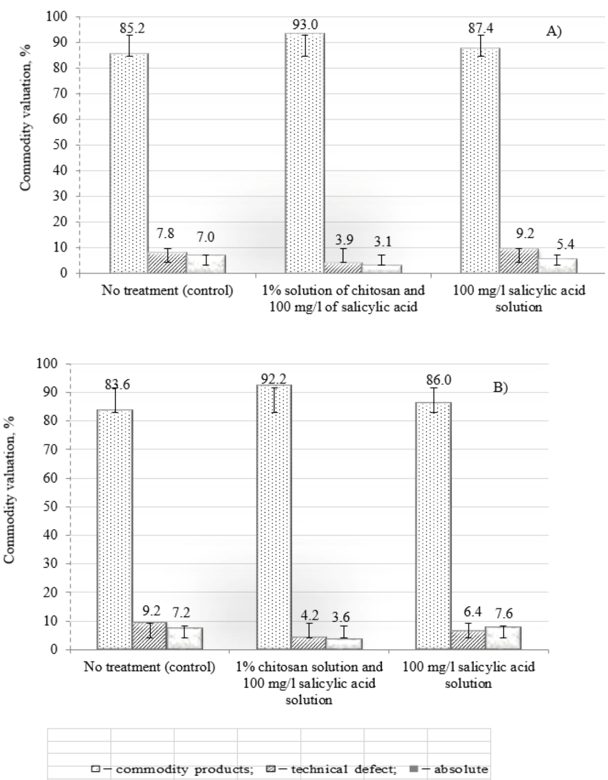

Figure 1: Output of marketable produce of sour cherry fruit 'Alfa' (A) and 'Pamiat Artemenko' (B) after storage (LSD_{05} of commodity produce = 2.6; LSD_{05} of technical defect = 0.2; LSD_{05} of absolute waste = 0.2).

Hence, the pre-storage treatment of sour cherry fruit with 1 % chitosan and 100 mg l^{-1} of salicylic acid increases the duration of their storage to 30 days, enhances the output of marketable produce by 7.8–8.6 %, reduces the level of technical defect by 3.9–5.0 %, and absolute waste – by 2.3 times as compared with mass losses which do not exceed 3 %.

The results of tasting evaluation of the sour cherry fruit, treated with polysaccharide compositions after storage, are shown in Fig. 3. After storage sour cherry fruit were evaluated by the following indicators: appearance, consistence, taste, scent, color.

By appearance, treated fruit were much better than the control, and they had excellent indicators. By scent, such differences were not recorded, except for the fruit treated with salicylic acid; they had a much lower tasting estimate – 4.5 points. The fruit treated with chitosan in combination with salicylic acid had an excellent scent.

Fruit color after harvesting was natural for sour cherry fruit. Significant differences from the control indicators and an excellent tasting evaluation was recorded on the sour cherry fruit treated with 1 % chitosan with 100 mg l^{-1} of salicylic acid. Contrary to this, the sour

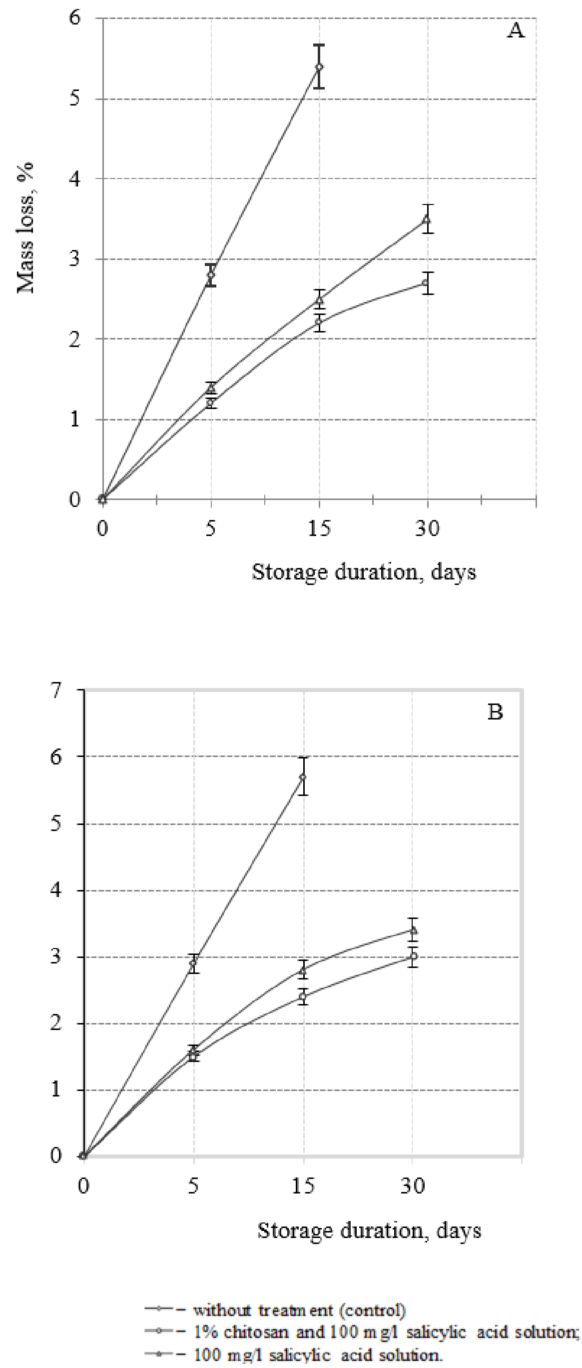

Figure 2: Mass losses of sour cherry fruit 'Alfa' (A) and 'Pamiat Artemenko' (B) during storage (LSD_{05} = 0.4).

cherry fruit treated with salicylic acid alone almost did not differ from the control – 4.5 points.

Along with color, another important indicator of product quality is consistence which plays a decisive role in choosing fruit for consumers. Softening occurred in the untreated sour cherry fruit (the control) after stor-

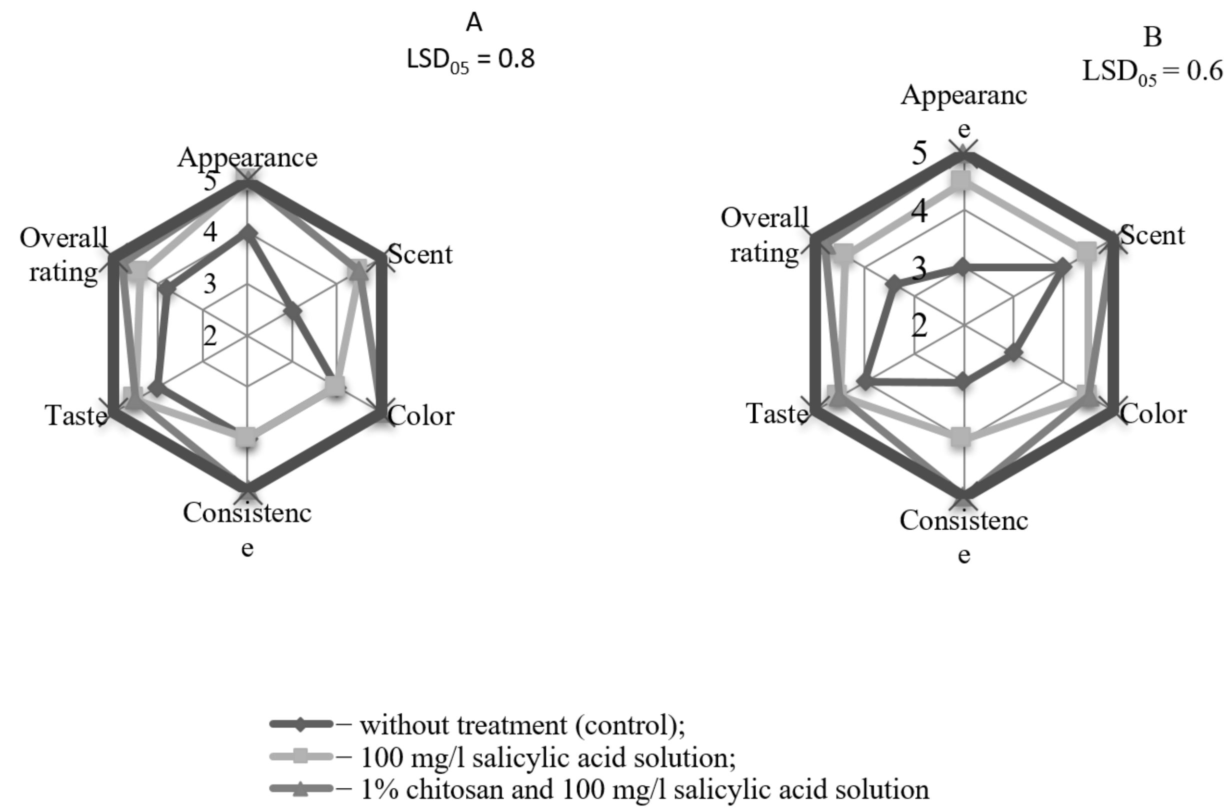

Figure 3: Organoleptice valuation of the sour cherry fruit 'Alfa' (A) and 'Pamiat Artemenko' (B) pretreated with polysaccharide compositions at the end of storage, 2016–2019.

age; this was caused by the worsening of a cell wall composition, due to pectin hydrolysis where such enzymes as polygalacturonase and pectinesterase were present. Similar findings were recorded in strawberry fruit during storage (Tomadoni et al., 2019).

For the sour cherry fruit which were pretreated with the combination of chitosan and salicylic acid, fruit consistence differed significantly from the control and it was evaluated in 5 points. Whereas the fruit treated with the solution of salicylic acid had a good tasting estimate (4 points).

The most important indicator of product quality is its taste. After storage, untreated sour cherry fruit had good taste properties (4 points). The evaluation grade of the fruit treated with the combination of chitosan and salicylic acid differed significantly. Namely, the sour cherry fruit treated with this solution had an excellent tasting estimate. It can be explained by the fact that chitosan can most likely add some taste to cherry fruit, which in turn influences their tasting estimate (Tomadoni et al., 2019).

Thus, the pre-storage treatment of sour cherry fruit with a polysaccharide composition of chitosan with salicylic acid appears to be the most efficient one due to the preservation of organoleptic properties of the produce at the level of fresh fruit and an excellent tasting estimate.

The complication in the organization of juicy fruit protection during their storage is defined by not only their mechanical structure, chemical composition and organoleptic indicators but also by the lack of active protective responses from plants themselves.

Microscopic fungi are the reason why diseases occur during juicy fruit storage. They can be classified into two groups – parasitic (phyto-pathogenic) and saprotrophic. The examples of such fungi are monilios carriers (*Monilinia fructigena* Honey and *Monilinia laxa* (Aderh. & Ruhland) Honey), grey mold (*Botrytis cinerea*), leaf browning (*Schizothyrium pomi* (Mont. & Fr.) Arx,), alternarios (*Alternaria* spp.).

Saprotrophic fungi cannot infect live healthy plant and they usually inhabit fruit after harvesting (during their transportation and storage). The example of such fungi are molds which are caused by some kinds of species *Aspergillus*, *Penicillium*, *Trichothecium*, *Mucor*, *Rhizopus* and others.

The most harmful disease is a bacterial disease caused by *Pseudomonas syringae* Van Hall. Fungus damage caused such kinds of species as *Penicillium, Botrytis* and M*onilia* leads to blue rot and grey mold. The occurrence of these rots and their effect on fruit, cherry fruit in particular, depend on varietal features and the ripening stage of the yields (Akulov, 2012).

Sweet and sour cherry fruit are damaged by various causative agents of species monilinia (*M. laxa, M. frutigena and M. fructicola* (G.Winter) Honey), blue mold, caused by *Pencillium expansum*, *Alternaria* and *Cladosporium* molds, caused by *Alternaria alternata* (Fr.) Keissl and *Cladosporium* sp. *Penicillium expansum;* they all result in yield losses and worse fruit quality (Wani et al., 2014).

Caused by fungus, *Pseudomonas syringae (*species *Monilia)* leads to brown rot. The occurrence of rot and its impact on sour cherry fruit depend on a cultivar and fruit ripeness degree (Wani et al., 2014).

To limit the development of phytopthogenic fungi on fruit, it is important to organize the control over them in the period of plant vegetation. The more efficient the control over them "in the orchard", the fewer of them will be during the fruit storage. To limit the development of saprotrophic fungi, it is important to minimize fruit damage. Besides, the fruit planned for a longer storage period must not be damaged by pests.

To improve the appearance of fruit and to slow down their spoilage, the procedure of putting an artificial wax-like layer on their surface is often done after harvesting the yield. This layer blocks gas exchange between the fruit and the atmosphere, and it also prevents water evaporation. Various fungicides are frequently applied right before or even during the smearing of this protective layer.

Such substances as salicylic acid, chitosan, and alginate are the inducers of the acquired system resistance of the plants to diseases, or they are used directly to protect cherry fruit from diseases during their storage (Akulov, 2012).

Chitosan is a safe alternative to synthetic fungicides against *B. cinerea* in strawberry (Petriccione et al., 2015). Disease *Botrytis* (grey mold) and strawberry rotting begin on the 6th day of their cold storage both on untreated fruit and those treated with 1 % chitosan covering. The strawberry fruit, covered with 1.5 and 2 % solution of chitosan were affected by microbiological spoilage only on the 9th day (Nasrin et al., 2017).

It was established (Tokatlı & Demirdove, 2020) that the total quantity of mesophylic aerobic bacteria in sweet cherry fruit, covered with chitosan, was smaller than expected, whereas it was equal to 2.74 log CFU g^{-1} at 4 °C in the untreated control group.

The research of the treatment effect with the solutions of polysaccharide compositions on micro-flora of sour cherry fruit after storage showed that the average amount of epiphytic micro-flora in fresh sour cherry fruit was at the level 1.1·10^3 CFU g^{-1} (Fig. 4).

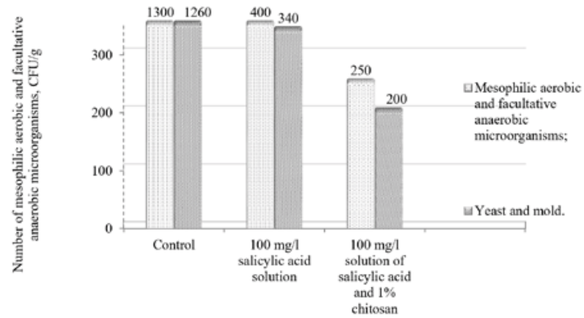

Figure 4: Effect of the treatment with the solutions of a polysaccharide combination on a quantitative and qualitative composition of epiphytic microflora on the surface of sour cherry fruit at the end of storage ($LSD_{05MAFAnM}$ = 17.5; $LSD_{05\ yeast}$ = 36)

After storage its amount increased up to 1,3·10^3 CFU g^{-1}. The treatment of sour cherry fruit with chitosan and salicylic acid decreased significantly the quantity of MAFAnM – by 5.2 times, as compared with the control. The decrease in the number of MAFAnM on sour cherry fruit, treated with the composition of chitosan and salicylic acid, can be explained by the availability of chitosan in it; and chitosan shows anti-bacterial properties. Positively charged amino-groups on chitosan bind to negatively charged carboxyl groups on a membrane of a bacterial cell, changing the distribution of a charge on a cell surface which causes the disturbance of membrane stability (Tokatlı &Demirdove, 2020).

The pretreatment of sour cherry fruit before storage led to the growth suspension of yeast and mold. In the control, they were at the level of 1.26·10^3 CFU g^{-1}; when fruit were treated with the solutions of polysaccharide compositions it decreased by 3.7–6.3 times. And the smallest amount of epiphytic micro-flora remained in sour cherry fruit treated with the solution of chitosan and salicylic acid.

Similarly, the decrease of the amount of yeast and mold on the cherry fruit, covered with aloe-vera, was recorded by D. Martinez-Romero et al. (2006). M. Rassa, M. Ghasemnezhad et al. (2013) stated that the treatment with 1% solution of chitosan reduces the growth of fungi, as compared with the control (Nasrin et al., 2017). After storage, monilios disease was mostly detected (it was caused by fungal flora of *Monilia* species).

Thus, the pre-storage treatment of sour cherry fruit with salicylic acid in combination with chitosan, ensures the decrease in the number of MAFAnM by 5.2 times, yeast and mold – by 6.3 times, as compared with the untreated fruit.

4 CONCLUSIONS

The pre-storage treatment of sour cherry fruit with 1 % solution of chitosan with 100 mg l^{-1} of salicylic acid extends the duration of their storage up to 30 days, enhances the output of marketable produce by 7.8–8.6 %, decreases the level of technical defect by 3.9–5.0 %, and absolute waste – by 2.3 times as compared with mass losses which does exceed 3 %.

The pretreatment of sour cherry fruit with polysaccharide composition of chitosan with salicylic acid before storage appears to be the most efficient technique due to the preservation of organoleptic properties of the produce at the level of fresh fruit and an excellent tasting estimate.

The treatment of sour cherry fruit with salicylic acid in combination with chitosan before storage results in the decrease of the quantity of MAFAnM by 5.2 times, yeast and mold – by 6.3 times, as compared with the untreated fruit.

Taking into consideration a high percent of the fruit spoilage after harvesting, during transportation and storage, it is believed to be promising to continue further research aimed at studying the effect of postharvest treatment with food films on their quality during storage.

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