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EFFECT OF CHITOSAN PRETREATMENT ON THE QUALITY OF STRAWBERRIES DURING COLD STORAGE

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Abstract. The paper is focused on improving the technology of storing strawberries. It has been investigated how pretreatment of berries with aqueous solutions of low-molecular-weight chitosan of three concentrations (0.1%, 0.3%, 0.5%) affects the quality parameters of strawberries during refrigeration. The treated berries and the reference (untreated sample) were stored in 500 g perforated plastic containers at 0±2°C for 14 days. It has been found that strawberries treated with chitosan solutions had significantly smaller weight loss than the reference. At the end of storage, this parameter was 9.7% in the reference and 7.0–8.6% in the treated berries. It has been established that the respiration rate of the strawberries decreased sharply on the first day, which was caused by refrigerated storage, and continued to decline until the end of storage. Finally, this parameter attained the value 3.3 mg $CO_2/kg^{-1}h^{-1}$in the reference and 2.2–3.0 mg $CO_2/kg^{-1}h^{-1}$ in the treated berries. The hardness of the strawberries at the end of storage was 0.10–0.14 kg/cm^2. The change in the lustre level of the berries has been observed. It has been established that on the 14th day of storage, the surface of the untreated berries was dull. The best characteristics have been observed for treatment at the chitosan concentration 0.5%. The effect of chitosan films on the sensory characteristics of berries has been investigated. It has been found that the pretreatment did not impair the taste of the berries. The results of the tasting evaluation indicate that the taste, aroma, and colour were better in the variants with the treatment concentrations 0.3 and 0.5%. However, as for the appearance and consistency, the experts preferred the berries treated at the concentration 0.5%. After two weeks' storage, the strawberries have been found to be damaged by four fungal diseases. The infections found in the samples were *Botrytis cinerea (*grey mould), *Rhizopus stolonifer (*black mould), *Whetzelinia sclerotiorum* (white mould), *and Penicillium spp*. It has been established that pretreatment of strawberries with chitosan solutions reduces the development of phytopathogenic diseases. It has been shown that chitosan-based edible coatings have a positive effect on strawberries, increasing their shelf life and improving their quality. A conclusion has been drawn about the technology of application of chitosan solutions and about their concentrations.

Keywords: strawberry, chitosan, cold storage, quality.

Introduction. Formulation of the problem

Strawberries belong to the most common and important fruits in the world, widely investigated for their nutritional and nutraceutical properties [1]. However, in recent years, the microbiological safely of fresh strawberries have been a matter of growing concern associated with fungal nutritional diseases [2].

Strawberry fruit are particularly perishable, and when harvested, they can suffer from desiccation, quality deterioration, and mould. The main postharvest decay factor is grey mould, followed by black mould, blue mould, and mucor, which are caused by *Botrytis cinerea*, *Rhizopus stolonifer*, *Penicillium spp.*, and *Mucor spp.* respectively [3]. Besides microbiological damage, strawberries quickly lose their appearance during storage, and this results in significant inventory and financial losses.

Analysis of recent research and publications

Strawberry (*Fragaria ananassa*) is a highly perishable fruit with a limited postharvest life at room temperature. It is vulnerable to postharvest decay due to its high respiration rate, environmental stresses, and pathogenic attacks [4].

The health benefits of strawberries are well-known. Strawberries play an important role in human nutrition, and they are a valuable fruit in our diet. Strawberries are low in calories (32 cal/100 g) and fats, but are a rich source of health-promoting phytonutrients, minerals, and vitamins that are essential for health. Moreover, fresh berries are an excellent source of vitamin C (100 g provides 58.8 mg or approximately 98% of recommended dietary allowance), as well as of A, E, and B-complex vitamins (the latter are also a powerful natural antioxidants) [5].

Due to fast metabolic activity, heavy loses occur in strawberries before they reach consumers. Besides, the postharvest diseases of strawberry fruit cause considerable losses during storage and transportation [6].

The main danger to fresh berries is phytopathogenic damage, which is caused by the development of various fungal diseases. Management of postharvest diseases is based mainly on chemical control, but fungicide applications can cause some complications such as toxic residues on the fruits and the variety of resistant isolates of the pathogen [7].

Now, one of the promising methods of protecting berries from damage and extending the shelf-life after harvesting is applying edible biopolymer-based coatings on the fruit's surface.

Edible coatings and films consist of natural polymers, that can improve the quality and safety of food by providing selective barriers to moisture transfer, oxygen uptake, and lipid oxidation, and by acting as carriers of antimicrobial agents that impart antimicrobial properties to films and coatings [8,9]. These films and coatings have recently gained interest in the field of food preservation. Chitosan-based edible films and coatings have appeared to offer a good prospect of their application in food preservation [10].

Edible films applied on vegetable and fruit raw materials are thin layers of substances, mostly polysaccharides, oils used in the food industry as a safe way to combat fungal diseases and extend shelf life [11-16].

Analysis of literature sources has revealed that the authors positively characterise the following substances: chitosan-based solutions; sodium alginate solutions, and pullulan solutions as an effective means to prevent the development of phytopathogenic damage. It is known that films based on sodium alginate and combined with it have an antibacterial effect and are able to improve the overall quality of fruit and vegetables during storage [17-22].

Sodium alginate is a polysaccharide consisting of residues of D-mannuronic and L-guluronic acids. Sodium alginate is obtained from red and brown algae and is used in the food industry as an emulsifier, stabiliser, gelling agent, and film former [23-27].

In 2019, studies were conducted on the effect of sodium alginate on the shelf life and quality of strawberries. Scientists have found that pretreatment of berries with 1%, 2%, and 3% of sodium alginate solution helped to preserve total acidity, vitamin C, phenol, and anthocyanin [28]. In the same year, another group of scientists proved that sodium alginate films were effective in combating fungal diseases [29], and T. Senturk Parreidt investigated that such coatings could significantly reduce the weight loss of berries during storage [30].

Pullulan is a water-soluble non-toxic food biopolymer that has film-forming and adhesive properties. Pullulan films are transparent, tasteless, and odourless [31-33]. A group of scientists from China concluded that pullulan films or films in combination with pullulan could be an alternative to synthetic means of storing fruit and vegetables. They proved that such coatings will better preserve the content of soluble dry matter and titratable acids in strawberries [34].

Pullulan is a microbial exopolysaccharide formed from *Aureobasidium pullulans* by deep fermentation of a medium containing carbon, nitrogen, and other nutrients. These nutrients are expensive, which significantly increases the cost of pullulan production [35].

Edible films and coatings based on chitosan have shown great potential for their use in the food storage technology. Chitin and chitosan are natural polysaccharide polymers. These polymers are used in several areas of agriculture, in food and food additives [36-40].

Chitosan is a polysaccharide derived from chitin, which is commonly found in the shells of insects and crustaceans, as well as in the cell walls of some fungi. It is known as the second most common biopolymer in nature after cellulose [41-45]. With the use of refrigerated storage, such films are effective in extending shelf life. Treatment with chitosan-based substances is used both before and after harvesting.

Due to its biocompatibility, chitosan combines well with various substances and has a positive effect on the quality of agricultural products [46-48].

According to M. A. Ibrahim, chitosan goes well with the essential oils of lemongrass and thyme. The films with these essential oils have been found effective in improving the quality of strawberries and extending shelf life to 15 days [49].

In the literature, there are publications where the authors claim that solutions of chitosan or ones used in combination with chitosan have a positive effect on the physicochemical properties of strawberries during storage [50-55].

Badawy *et al.* found that food films with chitosan delayed changes in the content of anthocyanins, soluble solids, and also had an effect on the inhibition of enzymes that destroyed cell walls [56].

In 2019, Brazilian scientists proved the effectiveness of treating berries with chitosan dissolved in acetic acid. They found that such coatings could slow down metabolism and delay the loss of weight, CPP, sugars, and ascorbic acid [57].

Berries coated with 1% and 2% chitosan solution delayed the breakdown of polyphenols, anthocyanins, and flavonoids. Besides, chitosan films enhanced the activity of some enzymes, preventing the softening of strawberry tissues and reducing membrane damage [58]. Chitosan coatings were recognised as an effective way to delay a pH increase [59].

Analysis of literature sources has shown the need for a detailed experimental study of how pretreatment of berries with aqueous solutions of low-molecular-weight chitosan affect the quality of strawberries.

The purpose of this work was to study the effect of pretreatment of strawberries with a chitosan solution before storage by determining the physical, physicochemical, organoleptic, and microbiological parameters. The **objectives** of the study:

- to investigate the changes in the weight loss of strawberries,
- to investigate the changes in the intensity of respiration of strawberries,
- to investigate the change in the tissue hardness of strawberries,
- to investigate the change in the lustre level of strawberries,
- to establish the effect of chitosan on the sensory properties,
- to determine the antibacterial properties of chitosan.

Research materials and methods

Strawberries of the consumer degree of ripeness (*Fragaria ananassa*) of the variety Dukat were obtained from the field of the Uman National University of Horticulture at the end of May 2019. Chitosan $(C_6H_{11}NO_4)_n$ with low molecular weight (50,000Da; 75% deacetylation), were purchased from Sigma-Aldrich Co. (St.Louis, MO).

To obtain aqueous solution, we weighed chitosan, covered it with distilled water at 40–45ºC, and left it for 18 hours (normal conditions) for swelling (for example, to obtain an aqueous solution with the concentration 0.3%, 3 g of chitosan was weighed and covered with 997 ml of distilled water), after which the mixture was slowly brought to 60°C using an electric heater and kept for 45 minutes, being stirred continuously. After chitosan dissolved, the solution was cooled to 20–22°C and used to treat strawberries with. Strawberries were treated with chitosan solution by spraying, followed by removal of moisture by active ventilation, which took place in a refrigerator. The solution had the concentrations 0.1%, 0.3%, 0.5%. The dried berries were packed into 500 g plastic containers and stored in the refrigerator at 0±2°C for 14 days.

Weight loss. The berries were weighed before storage and once in every three days during storage. The weight loss was calculated as the difference of two weighings. The criteria that the storage ended was the weight loss no more than 10% in each of the samples, which differed in the chitosan concentration in the solution.

Determining the respiration rate. Strawberry samples weighing 200 g each were put into a desiccator for 2 hours, meanwhile generated CO_2 reacted in which there is always an alkali as a standard (20 ml 0.4 M NaOH). The respiration rate was determined by titration with 0.2 M oxalic acid and expressed as millilitre CO_2 Kg^{-1} (fresh mass) per hour. The hardness of the berries was determined with a penetrometer (FT 02). Three repetitions were carried out for each sample.

Sensory characteristics. The sensory characteristics of strawberries (taste, appearance, texture, colour, aroma) were determined by tasting. The evaluation was performed using a 5-point scale. Changes in the lustre were measured visually on a 5-point scale, where 1 signified a dim berry surface without lustre, and 5 meant a shiny, glossy surface.

Microbiological damage was determined using a microscope MICROmede XS–2610 (fiftyfold magnification), taking samples at the end of storage with a bacteriological loop.

The lustre level and degree of microbiological damage of the berries were determined once at the end of the shelf life.

Phytopathogenic damage was determined by microscopy. Unlike the standard method of determination by washing, the microscopy makes it possible to determine quickly and accurately the type of fungus by its structure.

Results of the research and their discussion

Strawberries are high in moisture, which is lost through thin integumentary tissues due to rapid physiological changes. Weight loss in berries during storage is due to the relatively high intensity of respiration, reduced nutrient content, and development of phytopathogenic damage [60].

Studies have shown the effect of chitosan treatment on weight loss during storage (Fig. 1). As a result, strawberries treated with chitosan solutions had significantly smaller losses than the reference samples did. The weight loss in berries is caused by the loss of moisture and the continuation of the breathing process. After storage, the weight loss of the treated berries was by 1.1–2.7% smaller than in the reference. The results are consistent with the those of Tavares. The weight loss was more pronounced in the fruit of the reference treatment than in those observed in the fruit of other treatments. A faster rate of weight loss during storage of fruit was observed in the reference (1.15% a day), compared with the chitosan-treated fruit [61].

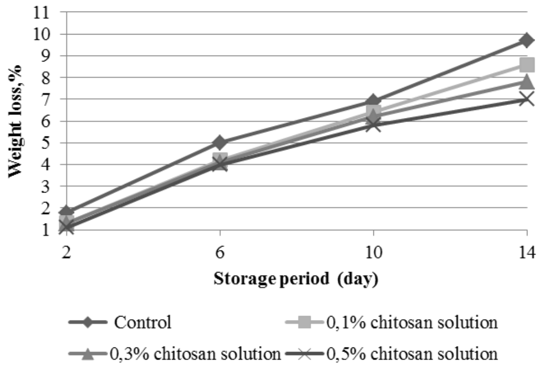

Fig. 1. Weight loss of strawberries during storage

Respiratory rate is the main indicator of metabolic processes in berries. It is mainly a reduction in the respiratory rate that allows extending the shelf life of fruit [62]

Pretreatment of strawberries with chitosan solutions has a positive effect on reducing the respiratory rate (Fig. 2). After the strawberries were collected, their respiratory rate was 33.5 mg CO_2/kg^{-1} h^{-1}. On the second day of storage at 0±2°C, the respiratory rate decreased sharply to 10.0–11.0 mg CO_2/kg^{-1} h^{-1} in the treated berries and reference samples. This sharp decrease in the respiratory rate is explained by the stress berries undergo when cooled. By the sixth day and by the end of the storage period, the respiratory rate continued to decrease slowly. This confirms that chitosan-based films are able to slow down the respiration of berries.

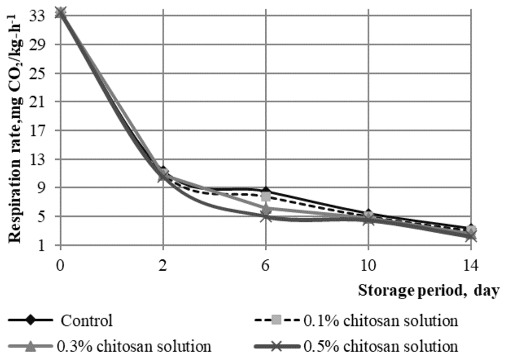

Fig. 2. Changes in the respiration rate of strawberries during storage (n=3, p<0.05)

The hardness of the tissues depends largely on the ripeness of berries. It varies with the variety of fruit, their size, and weather conditions during cultivation. High hardness contributes to better storage and transportation of raw fruit [64]. The strawberries to be stored were harvested at the consumer stage of ripeness. During storage, the tissue hardness was greatly reduced due to water loss and a high respiratory rate (Fig. 3). On day 14, the hardness of strawberries was 0.10, 0.12, and 0.15 kg/cm^2 (0.1, 0.3, and 0.5% chitosan solution respectively), which is by 0.02–0.05 kg/cm^2 more than in the reference. The chitosan-treated berries retained their hardness better than the untreated ones.

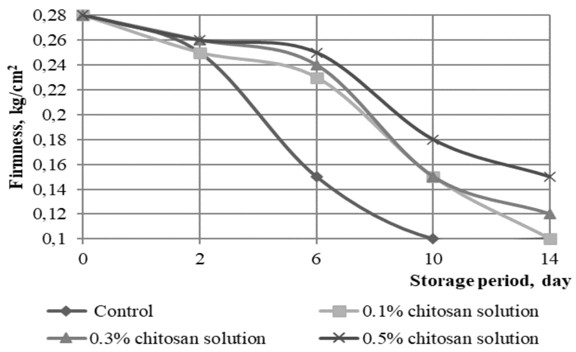

Fig. 3. Changes in the hardness of strawberries during storage

Organoleptic evaluation is one of the most important indicators of a product's quality. A potential buyer, first of all, pays attention to the appearance of the product, its colour, aroma, and texture. Preserving the natural attractiveness of berries is a complex process, because during storage, change of colour and loss of elasticity and aroma are inevitable. The strawberries treated with chitosan solutions had better sensory characteristics than the reference samples did (Fig. 4). According to the results of the tasting evaluation, the untreated sample was the worst. The experts noted that the colour of the berries was best preserved in the variants with the treatment concentrations 0.3 and 0.5%. As for the appearance, taste, aroma, and consistency, the sample treated at the highest chitosan concentration led the way.

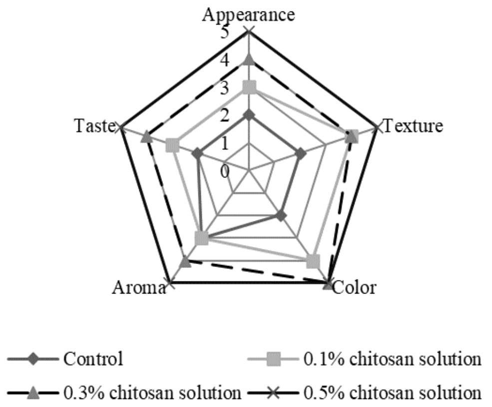

Fig. 4. Sensory characteristics of strawberries during storage

Lustre is the main criterion for the freshness of berries. This parameter is responsible for the external attractiveness of strawberries and partly determines their price. In all samples, at the beginning of storage, there was a slight loss of lustre (Table 1), but after 6 days of storage, the surface of the berries became dull. After storage, the best lustre was observed in the sample with the chitosan treatment concentration 0.5%.

Microbiological damage. Microbiological damage of strawberries is represented by 4 types of fungal diseases. Rot was detected during storage on the strawberries without chitosan pretreatment (fig. 5).

Table 1 – Change in the lustre of strawberries during storage

Sample	Storage period (day)				
	0	**2**	**6**	**10**	**14**
Reference	5	3	2	2	2
0.1% chitosan solutions	5	4	3	2	2
0.3% chitosan solutions	5	5	4	4	3
0.5% chitosan solutions	5	5	5	5	5

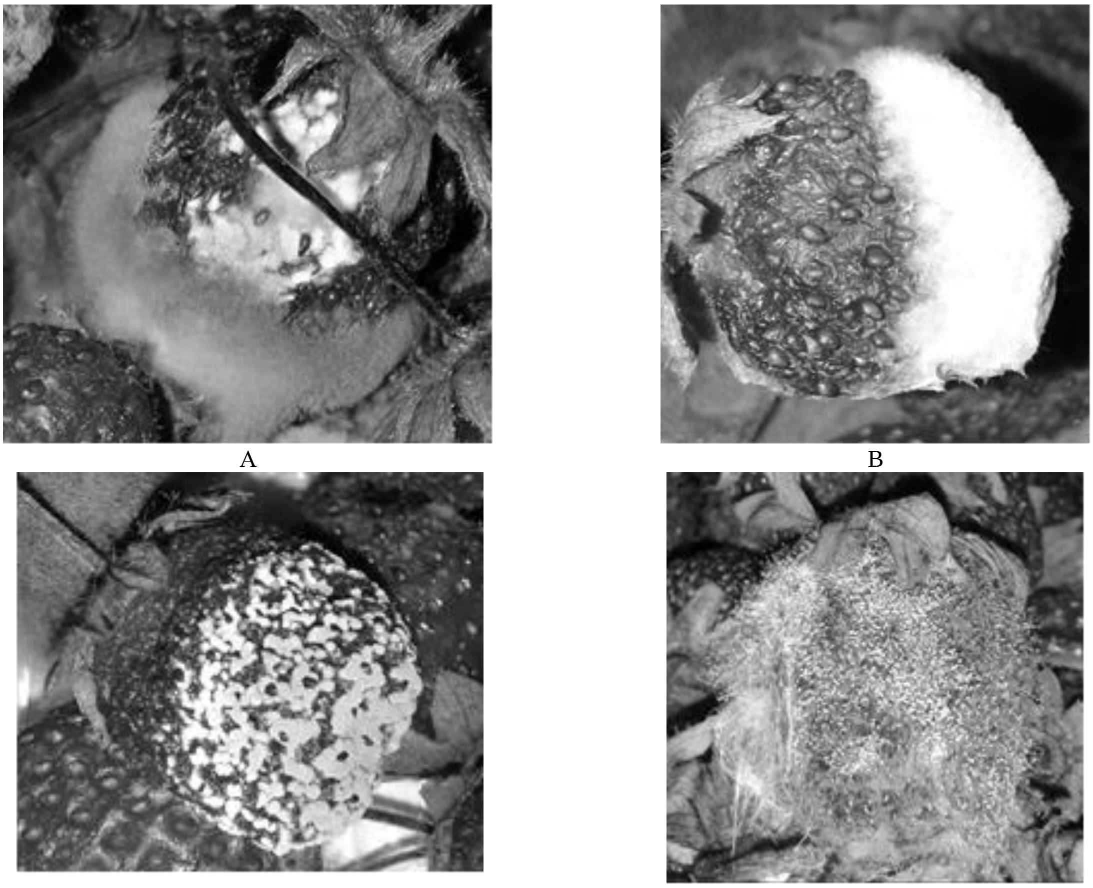

Fig. 5. Microbiological damage of strawberries:
(A) grey mould (*Botrytis cinerea*); (B) white mould (*Whetzelinia sclerotiorum*);
(C) blue mould (*Penicillium spp.*); (D) black mould (*Rhizopus stolonifer*)

The development of rot was recorded on the 14^{th} day of storage. As a rule, infection of berries starts on the mother plant and gradually develops during storage. The weather conditions also contributed to the infection. In May 2019, it was warm and humid. The total precipitation was 35.5 mm, the average temperature 19.2°C, the relative humidity 72%. Those weather conditions are favourable to the development of most fungal diseases. Phytopathogenic damage was not detected in the treated berries. These results indicate that edible coatings based on chitosan have antibacterial properties.

Conclusion

According to the results of the study, it can be concluded that pretreatment of strawberries before storage with aqueous solutions of low-molecular-weight chitosan has a positive effect on the product's quality. Chitosan coating has been shown to reduce weight loss by 1.1–2.7%, which is important for berries. It has been found that edible chitosan films reduce the intensity of respiration by 0.3–1.1 mg CO^2/kg^{-1} h^{-1}, allowing the berries to last longer. It has been investigated that the tissue hardness of the strawberries in the final composition ranged from 0.10 to 0.14 kg/cm^2.The degree of lustre of the berries has been determined. It has been found that on the 14^{th} day of storage, the surface of the berries without treatment became dull. The best results were observed in the variant with the chitosan treatment concentration 0.5%. It has been established that pretreatment did not impair the taste of berries. The results of the tasting evaluation show that the taste, aroma, and colour were better in the variants with the treatment concentrations 0.3 and 0.5%, but by the appearance and consistency, the experts preferred berries with the treatment concentration 0.5%. It has been found that the strawberries were damaged by four types of fungal diseases. *Botrytis cinerea* (grey mould), *Rhizopus stolonifer* (black mould); *Whetzelinia sclerotiorum* (white mould), and *Penicillium spp.* were detected in the reference samples. In the samples that had been pretreated with chitosan solutions, microbiological damage was not detected, which indicates the antibacterial properties of chitosan.

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ВПЛИВ ПОПЕРЕДНЬОЇ ОБРОБКИ ХІТОЗАНОМ НА ЯКІСТЬ СУНИЦІ ПІД ЧАС ЗБЕРІГАННЯ

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Анотація. Статтю присвячено вдосконаленню технології зберігання ягід суниці. Досліджено вплив попередньої обробки ягід водними розчинами низькомолекулярного хітозану трьох концентрацій (0,1%; 0,3%; 0,5%) на якісні показники суниці під час холодильного зберігання. Оброблені ягоди та контроль (варіант без обробки) зберігали у перфорованих пластикових контейнерах місткістю 500 грам за температури 0 ± 2°C протягом 14 днів. Встановлено, що ягоди суниці, оброблені розчинами хітозану, мали значно менші втрати маси, ніж у контролі. Після закінчення зберігання показник встановлюється на рівні 9,7% у контролі та 7,0–8,6% у оброблених ягодах. Досліджено, що інтенсивність дихання ягід суниці в перший день різко знизилася, що було викликано зберіганням в холодильнику і продовжувала знижуватися до кінця зберігання. В кінці терміну зберігання показник встановлений на рівні 3,3 мг CO_2/кг$^{-1}$ год$^{-1}$ у контролі та 2,2-3,0 мг CO_2/кг$^{-1}$ год$^{-1}$ у оброблених ягодах. Щільність ягід в кінці зберігання становила 0,10-0,14 кг/см^2. Визначено ступінь блиску ягід. Встановлено, що на 14-й день зберігання поверхня ягід без обробки була тьмяною. Найкращі показники спостерігалися у варіанті з концентрацією обробки хітозаном 0,5%. Досліджено вплив хітозанових плівок на органолептичні показники ягід. Було встановлено, що попередня обробка не погіршила смаку ягід. Результати дегустаційної оцінки свідчать про те, що смак, аромат та колір були кращими у варіантах із концентрацією обробки 0,3 та 0,5%, однак за зовнішнім виглядом та консистенцією фахівці віддали перевагу ягодам із концентрацією переробки 0,5%. Встановлено, що ягоди суниці при зберігання протягом двох тижнів були пошкоджені чотирма видами грибкових захворювань. У зразках виявлено *Botrytis cinerea* (сіра гниль); *Rhizopus stolonifer* (чорна гниль); *Whetzelinia sclerotiorum* (біла гниль) та *Penicillium spp*. Встановлено, що попередня обробка суниці розчинами хітозану зменшує розвиток фітопатогенних захворювань. Доведено, що їстівні покриття на основі хітозану позитивно впливають на ягоди суниці, збільшуючи термін їх зберігання та покращуючи їх якість. Зроблено висновок щодо технології застосування та концентрацій розчинів хітозану.

Ключові слова: суниця, хітозан, холодильне зберігання, якість.

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