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SHELF–LIFE CHARACTERISTICS OF EDIBLE–COATED NEW SWEET CHERRY CULTIVARS

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ХАРАКТЕРИСТИКИ СРОКА ГОДНОСТИ НОВЫХ СОРТОВ ЧЕРЕШНИ, ПОКРЫТЫХ СЪЕДОБНОЙ ОБОЛОЧКОЙ

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Two new Bulgarian selected hybrid sweet cherry cultivar candidates (El.17–90 "Asparuh" and El.17–37 "Tzvetina" — Fruit Growing Institute, Plovdiv, Bulgaria) and one standard cultivar (Bing) were chitosan treated and stored in refrigerator at 4 °C for 21 days, up to the endpoint of the experimental shelf-life time. Chitosan-Ca-lactate (multicomponent) and Chitosan-alginate (bi-layer) edible coating treatments were applicate in these experiments. The used coating formulas are bio compatibles, non-toxics and have antimicrobial activities. The sample series (five replicates with thirty fruits from each cultivar and each treatment, and a control) were inspected weekly based on the appearance. The healthy and intact fruits were tested for physical (visual sorting, weight loss and texture of the intact fruits), physico-chemical (refractometrical dry content, antioxidant activity, pH of the pulp), and microbiological properties (total number of microorganisms, *E. coli*, fungi and yeasts). At the beginning of the storage, the control samples (not treated) showed better appearance and harder texture than the treated samples, because they had to suffer less manipulations. Later they lost these preferences, but the Chitosan-alginate treat was able to preserve the fruits in a better state. This study shows that the edible coating is a promising method to preserve the nutrition value of fresh fruits. The application is possible in preparation of ready-to-eat fruit salads or in fruit decoration of confectionery products.

Keywords: Physical properties, sweet cherry hybrids, Chitosan treatment

Аннотация. Плоды двух новых болгарских гибридных сортов черешни (кандидатов на сорт) Ел. 17–90 «Аспарух» и Ел. 17–37 «Цветина» (селектированные Институтом плодоводства, Пловдив, Болгария) и один стандартный сорт «Бинг» обрабатывали хитозаном и хранили в холодильнике при 4 °С в течение 21 дня до конца экспериментального срока годности. Плоды обрабатывали съедобными смесями: многокомпонентным хитозан-Са-лактатом и двухслойным хитозан-альгинатом. Используемые формулы покрытия являются биосовместимыми, нетоксичными и обладают антимикробной активностью. Серия образцов (пять повторностей с тридцатью плодами от каждого сорта и каждой обработки плюс контрольный образец) проверялась еженедельно на основании внешнего вида. Здоровые и неповрежденные плоды были проверены на физические характеристики (визуальная сортировка, потеря веса и текстура неповрежденных плодов), физико-химические (содержание сухих веществ по рефрактометру, антиоксидантная активность, pH пульпа) и микробиологические свойства (общее количество микроорганизмов, Е. коли, грибки и дрожжи). В начале хранения контрольные образцы (не обработанные) показали лучший внешний вид и более твердую текстуру, чем обработанные образцы, поскольку им приходилось меньше подвергаться манипуляциям. Позже они потеряли эти предпочтения, в то время как хитозан-альгинатное покрые смогло сохранить фрукты в лучшем состоянии. Это исследование показывает, что нанесение съедобного покрытия на свежие фрукты является многообещающим методом сохранения их питательной ценности. Применение возможно при приготовлении готовых к употреблению фруктовых салатов или при фруктовом оформлении кондитерских изделий.

Ключевые слова: физические свойства, гибридные сорта черешни, обработка хитозаном.

Introduction

Sweet cherry (*Prunus avium* L.) is amongst the most commercially important *Prunus* fruit tree species planted in temperate climate zones. In the context of substantial changes to environmental conditions induced by climate change, it will be essential that plant cultivars are well adapted to warmer winter and spring temperatures and to more extreme climatic events such as erratic spring frosts and summer heat waves. This is especially true for perennial fruit crops, which require more than a decade before a new cultivar is released. The sweet cherry fruits are very sensitive for quality loss with short ripening season and after harvesting, during transport or shelf-life maintenance [1]. Sweet cherry is a major structural species in Bulgaria [2].

In the recent years, the interest is growing for the ready to eat healthy foods, fruit-salads and desserts. In this argument the edible coating is a useful technology, because it is environmentally friendly, and helps to preserve the freshness and nutrition value of the fruits [3]. Chitosan is a natural carbohydrate polymer consisting of (1,4) — linked 2-amino-deoxy-β-d-glucan (cationic) obtained by the deacetylation of chitin from shrimp or fungal waste. It is often tested for edible coating in recent years due to its potential for industrial applications. The acid or water solubility of chitosan depends on the acetylation degree and the molecular weight [4]. The semi-permeable chitosan films are durable, flexible and easily tearable, decrease transpiration loss, delay the ripening of fruits, inhibit the growth of microorganisms [5]. The Ca-lactate modify the texture preservation effect of the coatings and increase the extension of the shelf-life [6]. The alginate is a gel-forming anionic polysaccharide, which is also often used in edible coatings [7]. The alginate layer can increase the oxygen barrier effect of chitosan [8]. The alginate coatings improve the quality and shelf-life of different minimally processed fruits, such as apples [9], papayas [10], and pineapples [11].

There are limited studies in the literature that relate to coating sweet cherries with chitosan-Ca-lactate like multicomponent or Chitosan-alginate like layer-by-layer coatings. Dang [12] found that chitosan coating on cherries, especially those at the 3 and 5 g/L concentrations, retarded loss of water, titratable acidity, and ascorbic acid, increased the ratio of total soluble solids and titratable acidity.

The objectives of the present study were to evaluate and compare the effects of water-soluble chitosan-Ca-lactate and water-soluble chitosan-alginate coatings on different sweet cherry varieties during refrigerated storage at 4 °C.

Materials and methods

1. Materials

Sweet cherry (*Prunus avium L.*) cultivar candidates (El. 17–90 "Asparuh" and El. 17–37 "Tzvetina" from the Fruit Growing Institute, Plovdiv, Bulgaria) and one traditional cultivar ('Bing') were chitosan treated and stored in refrigerator at 4 °C for 21 days, up to the endpoint of the experimental shelf-life time. The breeding program and the cultivar candidates were described by Malchev & Zhivondov [2]. The used cultivars were chosen based on the very similar ripening period. Chitosan-Ca-lactate (multicomponent) and Chitosan-alginate (bi-layer) edible coating treatments were applicate in these experiments. The food-grade, water-soluble chitosan was purchased from Xi'an Lyphar Biotech Co., LTD, China. Also the food grade Ca-lactate and the sodium alginate was bought from Sigma Aldrich, Bulgaria. The end point of shelf-life time was inspected based on the visual loss.

Used treatments:

1. Control: washed and selected intact fresh fruits. On the 0^{th} day just the control samples were analyzed.

2. Chitosan-Ca-lactate (multicomponent): Control + immersed in 0.01 $kg.kg^{-1}$ low molecular weight chitosan + 0.01 kg. kg^{-1} calcium-lactate*$5H_2O$ solution for 10 min and dried for 10 min intact fresh fruits.

3. Chitosan-alginate (bi-layer): Control + immersed in 0.01 $kg.kg^{-1}$ low molecular weight chitosan solution for 10 min., dried for 10 min. + immersed in 0.01 $kg.kg^{-1}$ sodium alginate solution for 10 min. and dried for 10 min. intact fresh fruits. Layer-by-layer technology based on the electrostatic deposition of the polycation chitosan and the polyanion alginate to coat fruit bars enriched with ascorbic acid.

During the 21 days storage times 30 probes from each treatments were analyzed 3 times (weekly) based on the visual appearance and loss of physical, physico-chemical and microbiological properties.

2. Physical properties:

Visual quality loss: the healthy, intact fruits were chosen for the experiments, and it is given in% for the actual 30 pieces on tray.

Weight loss: each control and treated fruit was weighed in each time, after the visual selection and before any other experiments. The weight loss was expressed as the percentage loss compared to the initial weight.

Texture: 1/3 of the selected fruits were tested by rupture test with (probe cylinder d = 5 mm, test speed v = 1 $mm.s^{-1}$) SMS TA XT2+ texture analyzer. Yield point (Young modulus, yield force and yield deformation, deformation work) and rupture point (rupture force and rupture deformation) parameters were used for statistical analyzing.

3. Physico-chemical properties:

For the purpose of the physico-chemical properties equalized fruit-pulp was prepared from 1/3 of the selected fruits.

Soluble solid content was expected by ABBE type refractometer at 20 °C in five repetitions. The results is presented as percentage (°Brix). (BDS EN ISO 12143:2000)

Antioxidant activity: Total antioxidant activity (TAA) was quantified by the method based on the capacity of different components to scavenge the ABTS radical cation compared to the standard antioxidants (ascorbic acid and Trolox) in a dose response curve. TAA due to both hydrophilic and lipophilic compounds in the same extraction. The results are expressed as mg of Trolox equivalent 100 g^{-1} [13].

Active acidity (pH) of the pulp was measured by a Microsyst pH meter at 20 °C in five repetitions. (BDS 11688:1993)

4. Microbiological methods:

The *total number of microorganisms* (TNM — BDS EN ISO 4833–2:2013), the *total coliform bacteria* (ISO 16649–2:2001) and the *total yeasts and molds* (TYM — BDS EN ISO 21527–2:2011) were detected based on the horizontal method for enumeration.

The results were expressed as a logarithm of colony forming units (log cfu/g) for *total number of microorganisms*, *total coliform bacteria* and *yeasts and molds*.

Results:

1. Physical properties:

Visual quality loss: During the storage, the amount of healthy fruits decreased. The decreasing depends on the cultivar and on the treatment as well. From candidate-cultivar "Asparuh" just the chitosan-alginate treated fruits had remained healthy to the end of the 3rd week (21 days). From candidate-cultivar "Tzvetina" both treatment and the control also had healthy fruits. From cultivar Bing all of the chitosan-Ca-L treated fruits were rotted, but from the control and the chitosan-alginate treated samples, the most fruits were preserved to the end of the 3 weeks period. (Fig. 1)

Weight loss: The weight loss of the cherry fruits means mainly water loss, because the fruit evaporates the water to the air [14]. The rate of water loss depends on the cultivar and on the coating as well. It was the largest for cultivar "Asparuh" and the smallest for cultivar "Bing". For cultivar "Asparuh" the used coating treatments could not decrease the water loss, just the quantity of the rotted samples. For cultivar "Tzvetina" the amount of weight loss at the beginning of the storage time is the same for the coated and the control samples, but at the last week it rises to the highest value for the control, for the chitosan-Ca-L is in the middle and it is the smallest for the Chitosan-Al coating.

For cultivar "Bing" with the chitosan-Al coating the loss of the weight is slower and there are some preserved healthy fruits to the end of the shelf-life. (fig. 2.) A part of the authors report similar effect for chitosan based coatings for sweet cherry and other fruits as well [15].

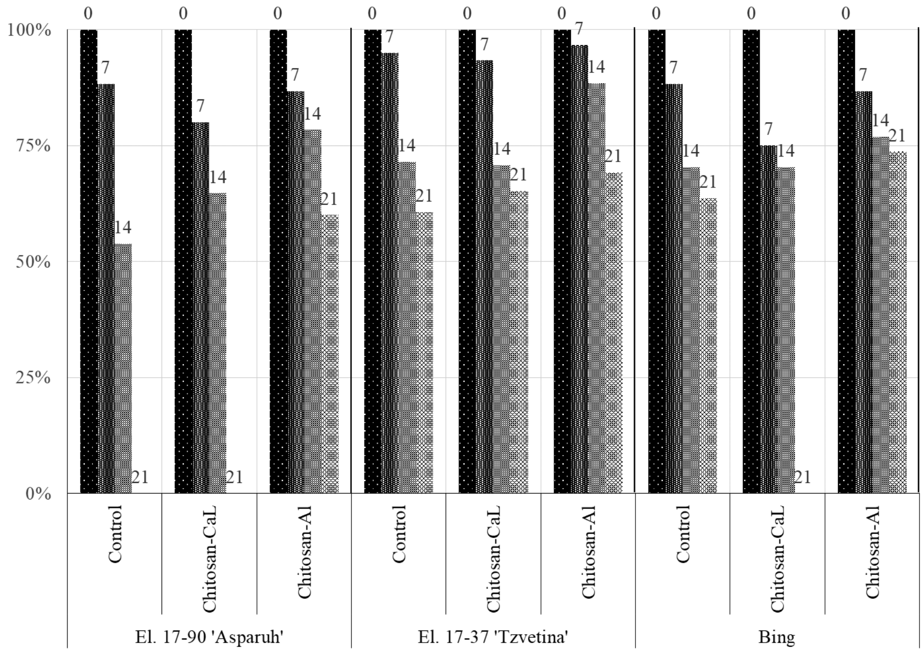

Figure 1. Result of the visual quality loss

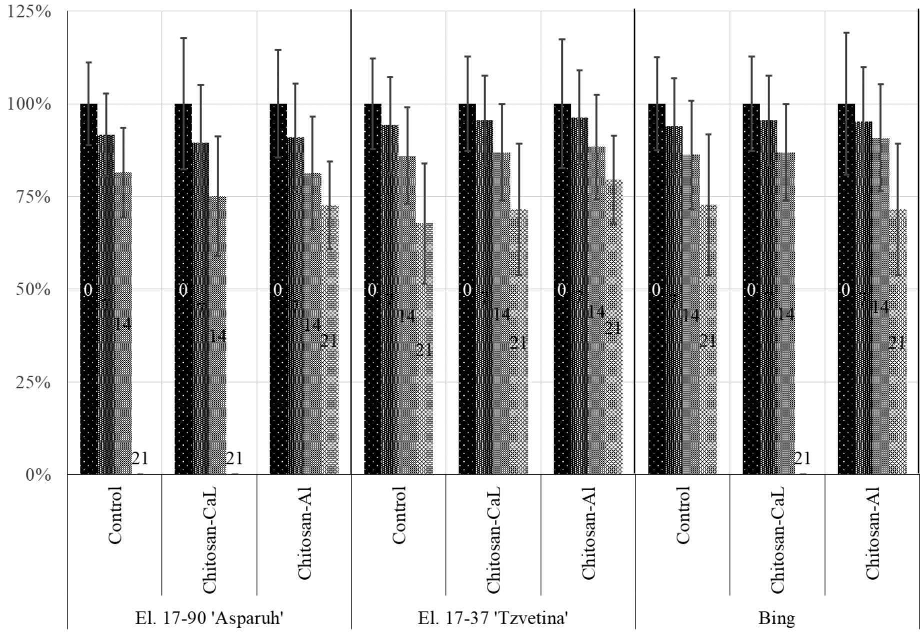

Figure 2. Result of the weight loss

Evaluating of the *texture parameters*:

Fruit texture is an important quality characteristics that influences the consumer acceptability. To explain better the texture changes, a combined, dimensionless parameter is able to be introduced, which is a ratio between the parameters of the rupture and yield points:

$$\text{Crunchiness} = \frac{F_f / F_r}{\ell_f / \ell_r}, \qquad (1)$$

where

F_f = yield force; F_r = rupture force; ℓ_f = yield deformation; ℓ_r = rupture deformation.

This parameter can explain the differences between a fresh fruit with high turgor (crunchy) and hard peel and flesh, and a stored, rotted fruit with softer peel and soft, but sticky flesh (mushy). The crunchiness shows the preserving effect of the used different edible coatings. The decreasing of this parameter is slower for the coated samples in all cultivars. At the beginning of the storage, the chitosan-CaLa coating showed higher values, but later this advantage ceases and the effect becomes the same like the other coating. Similar softening was reported in the study of Díaz-Mula [16].

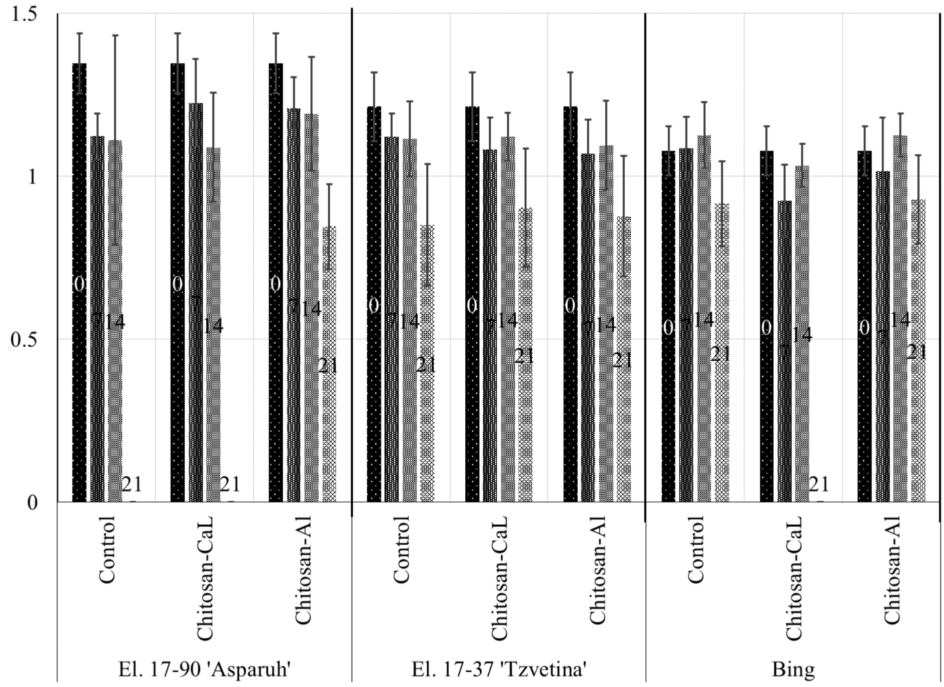

Figure 3. Results of the crunchiness

2. Physico-chemical properties:

Soluble solid content:

The non-treated fruits lost their water content (freshness). The chitosan-alginate treatment demonstrated better preservation of the soluble solid content for all varieties. The cultivars "Bing" and "Tsvetina" with Ca-Lactate treatment showed high level of increasing the soluble solid content (dried up). It means that this treatment has a good barrier effect only in a few cases. That result is very similar with the reported by some other authors [17, 18].

Antioxidant activity:

The antioxidant activity is decreased during the shelf-life. The used treatments in some cases could delay the decreasing, for e. g. for cultivar "Asparuh". This effect is analogous with reported in the study of Petriccione [18]. For cultivar "Bing" and "Tzvetina" the effect is not clear.

Active acidity (pH):

During the shelf-life, the pH of the fruits was increased. The chitosan treatments delayed the increasing in different scale. Although the levels of pH increasing were dependent on the cultivar like in the study of Aday, & Caner [15].

3. Microbiological properties:

The *total number of microorganisms* was decreasing at the beginning of the storage period, for the coated sweet cherry samples, but later it increased again. Finally, at the end the *total number of microorganisms* was almost the same or just slightly smaller than on the 1st day. That result is very similar to the result in Tokatlı [18].

Coliform bacteria (bellow 1 log cfu/g) in control and chitosan-coated sweet cherries were found to be below detectable levels during the storage periods. Very similar result was reported in Tokatlı [18] study for sweet cherry.

Coating sweet cherries with both chitosan combinations inhibited *yeast and mold* growth at 4 °C for 21 days. Yeasts and molds could have been detected just for the control samples. Martinez-Romero [19] reported that the total counts of yeast and mold were reduced in *Aloe vera* treated sweet cherries. Ghasemnezhad [5] coated pomegranate arils with chitosan and stored them for 12 days at 4 °C, and observed that treatment with 1% chitosan significantly reduced fungal growth compared with control.

Result of physico-chemical and microbiological properties

Cult.	Treat	D	Brix	Antioxidants	pH	TNM	TYM
El. 17–90 "Aspruh"	Cont.	0	14.2±0.2^{ab}	2709.46±1.38^{a}	3.78±0.02^{a}	5.22±0.1b	3.24±0.06
	Cont.	7	13.7±0.2^{a}	2426.45±0.51^{b}	3.98±0.01^{b}	4.28±0.07a	1
	Cont.	14	16.6±0.1^{b}	2378.37±0.80^{c}	4.12±0.02^{c}	5.47±0.02^{c}	2
	Cont.	21	n.a	n.a	n.a	n.a	n.a
	Cont.	0	14.2±0.2^{a}	2709.46±1.38^{a}	3.78±0.02^{a}	5.22±0.10^{b}	3.24±0.06
	Ch-Ca	7	15±0.2^{bc}	2582.47±0.58^{b}	4±0.01^{b}	4.02±0.02^{a}	1
	Ch-Ca	14	14.8±0.1^{b}	2505.22±0.38^{c}	4.09±0.02^{c}	5.47±0.01^{c}	2
	Ch-Ca	21	n.a	n.a	n.a	n.a	n.a
	Cont.	0	14.2±0.2^{a}	2709.46±1.38^{a}	3.78±0.02^{a}	5.22±0.10^{c}	3.24±0.06
	Ch-Al	7	14.8±0.10^{b}	2534.92±0.20^{b}	3.97±0.02^{b}	2.98±0.03^{a}	1
	Ch-Al	14	16.4±0.2^{d}	2484.48±0.20^{c}	4.08±0.01^{c}	5.53±0.05^{d}	2
	Ch-Al	21	15.3±0.1^{c}	2468.26±0.62^{d}	4.14±0.02^{d}	3.99±0.01^{b}	1
El. 17–37 "Tzvetina"	Cont.	0	18.3±0.2^{a}	2648.1±0.74^{a}	3.64±0.02^{a}	4.09±0.05^{a}	4.12±0.08
	Cont.	7	21.5±0.1^{bc}	2558.36±0.49^{b}	3.8±0.02^{b}	4.37±0.05^{b}	3.31±0.03
	Cont.	14	21.0±1.0^{b}	2502.32±99.44^{c}	3.98±0.02^{c}	5.02±0.02^{c}	4.47±0.04
	Cont.	21	22.6±0.1^{c}	2480.44±0.77^{d}	4.06±0.02^{d}	4.00±0.00^{a}	3.51±0.05
	Cont.	0	18.3±0.20^{a}	2648.10±0.74^{a}	3.64±0.02^{a}	4.09±0.05^{a}	4.12±0.08
	Ch-Ca	7	18.8±0.2^{a}	2577.52±0.86^{b}	3.97±0.02^{b}	4.25±0.04^{b}	1
	Ch-Ca	14	21.2±0.2^{b}	2545.52±0.60^{c}	4.18±0.02^{d}	6.47±0.05^{c}	2
	Ch-Ca	21	21.5±0.2^{bc}	2448.22±0.06^{d}	4.06±0.01^{c}	4.00±0.00^{a}	1
	Cont.	0	18.3±0.2^{a}	2648.1±0.74^{a}	3.64±0.02^{a}	4.09±0.05^{a}	4.12±0.08
	Ch-Al	7	21.0±0.2^{c}	2545.65±0.23^{b}	3.91±0.01^{b}	4.45±0.05^{b}	1
	Ch-Al	14	20.0±2.0^{b}	2521.51±0.28^{c}	3.95±0.02^{bc}	4.61±0.02^{c}	2
	Ch-Al	21	21.3±0.2^{c}	2489.81±2.08^{d}	3.99±0.01^{c}	3.99±0.01^{a}	1
Bing	Cont.	0	22.2±0.2^{a}	2886.65±0.70^{a}	3.71±0.02^{a}	4.87±0.02^{b}	4.83±0.01
	Cont.	7	23.7±0.2^{c}	2806.64±0.24^{b}	3.96±0.04^{b}	4.35±0.05^{a}	3.06±0.06
	Cont.	14	22.6±0.1^{b}	2779.27±1.01^{c}	4.2±0.1^{c}	5.19±0.07^{c}	3.96±0.02
	Cont.	21	22.4±0.2^{ab}	2707.97±0.25^{d}	4.42±0.01^{c}	4.85±0.01^{b}	4.63±0.03
	Cont.	0	22.2±0.2^{a}	2886.65±0.70^{a}	3.71±0.02^{a}	4.87±0.02^{b}	4.83±0.01
	Ch-Ca	7	26.2±0.4b	2787.22±0.54^{b}	3.83±0.02^{b}	4.05±0.06^{a}	2±0
	Ch-Ca	14	28.8±0.2^{c}	2707.22±0.25^{c}	3.9±0.03^{bc}	5.52±0.04^{c}	2±0
	Ch-Ca	21	n.a	n.a	n.a	n.a	n.a
	Cont.	0	22.2±0.2^{a}	2886.65±0.70^{a}	3.71±0.02^{a}	4.87±0.02^{c}	4.83±0.01
	Ch-Al	7	23.2±0.3^{bc}	2772.44±0.91^{b}	3.94±0.02^{b}	4.27±0.08^{b}	2
	Ch-Al	14	24.2±0.3^{c}	2656.18±0.23^{c}	3.96±0.01^{b}	5.54±0.06^{d}	2
	Ch-Al	21	22.8±0.2^{b}	2552±0.55^{d}	4.22±0.02^{c}	3.98±0.03^{a}	1

Conclusions:

Based on the physical properties, candidate-cultivar El.17–37 "Tzvetina" has the best storability based on the biggest number of healthy fruits left from this cultivar. The effect of the treatment also depends on the cultivar. The chitosan-alginate treatment is better in preservation of the freshness for all used cultivars. The results obtained in this study demonstrate the antimicrobial activity of the chitosan.

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