CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage, **http,//chimie-biologie.ubm.ro/carpathian_journal/index.html**

INFLUENCE OF EDIBLE COATING ON SHELF LIFE AND QUALITY OF SWEET CHERRY

Gabor Zsivanovits[1]✉, Stoil Zhelyazkov[1], Maria Momchilova[1], Dida Iserliyska[1], Diyana Aleksandrova[2]

[1]Institute of Food Preservation and Quality, Plovdiv, Bulgaria,
[2]Fruit Growing Institute, Plovdiv, Bulgaria
✉g.zsivanovits@canri.org

https://doi.org/10.34302/crpjfst/2021.13.2.9

Article history:
Received,
29 January 2021
Accepted,
22 May 2021

Keywords:
Chitosan;
Sensory evaluation;
Physical properties;
Antimicrobial effect;
Anti-browning effect.

ABSTRACT

Edible coatings are environment friendly materials for extension the shelf-life and preservation the nutritional value of fruits. In the present research, two different sweet cherry cultivars were treated with chitosan, chitosan Ca-lactate (multicomponent, mono-layer coating) and chitosan alginate (polyelectrolyte complex – bi-layer coating). Immersing technology was used to apply the coatings, ones for the mono-component, monolayer chitosan and for the multicomponent, monolayer chitosan Ca-lactate and twice for the polyelectrolyte complex – bilayer chitosan alginate. The fruit quality of control and coated samples was evaluated about refractometric, colorimetric, textural, antioxidant activity (DPPH), acidity, microbiological and sensory parameters during 21 days refrigerated storage. The coatings delayed the decay of the sweet cherry varieties in different scale. There were differences in browning, in texture changing, in sensory parameters and microbiological contamination as well. The chitosan based edible coatings extended the shelf-life period of sweet cherry varieties.

1. Introduction

A relatively new practice for maintaining the quality of fresh cherries after harvest is their packaging with edible coatings and films (Vargas et al, 2009). There is information in the literature on the use of edible chitosan coatings as a means of extending the shelf life of cherries. Chaovanalikit and Wrolstad (2004) reported improved quality (based on texture, dry matter, and acidity) for 60 days for cherries coated with a chitosan film stored at low temperature and high relative humidity (98%). The most detailed study on the parameters of cherries packaged in edible coatings was carried out by Aday et al. (2010). Edible coatings were prepared from 3% chitosan (low viscosity) in acetic acid solution with added glycerol as a plasticizer. The cherries are stored at a temperature of 20 °C and a relative humidity of 55%. During 11 days of storage, the authors reported a greatly reduced level of oxygen and an increased level of CO_2 for packaged cherries, ie. chitosan exhibits membrane properties and creates a modified atmosphere at the surface of the fruits. They also reported decreases of the breathing rate. The weight loss on the 9th day for the coated samples was 25%, against 49% for the control. Weight retention, ie. the preservation of the water content in the cherries is directly related to the better appearance and the preserved texture. There is also a tendency for a slower change in pH, dry content and acidity. These results indicate the good barrier properties of chitosan coatings and their ability to extend the shelf life of packaged cherries. In order to optimize edible packages of chitosan, in our earlier research water-soluble chitosan was used in lower concentrations. When applying a 1% solution of chitosan in combination with alginate and calcium lactate, the shelf life of packaged cherries can be extended to 21-25 days. The respiration is reduced and the influence of the barrier properties of the packaging is less pronounced. The extension of the shelf life depends not only on the composition of the coating, but also on the cherry's variety.

Sweet cherries (Prunus avium L.) and sour cherries (Prunus cerasus) are among the most important commercial species of Prunus fruit trees, which are grown in continental climates. The cherry is the main structural species in Bulgaria (Malchev & Zhivondov, 2016). Production of sour cherry in Bulgaria, is relatively limited. Cherries are highly perishable, non-climatic fruits. The destruction of cherries occurs with rapid weight loss, discoloration, softening (Bernalte et al., 2003). Their shelf life is shortened in case of loss of hardness, change in stem color, drying and they are not suitable for long storage. Maintaining lower fruit temperatures immediately after harvest leads to harder fruits with reduced rot and greener stems (Schick & Toivonen, 2002). In the context of the significant changes in environmental conditions caused by climate change, it is essential that crops are well adapted to warmer winter and spring temperatures and to more extreme climatic phenomena, such as variable spring frosts and hot summer. Cherry and sour cherry fruits are highly sensitive to quality loss during the short ripening period and after harvest, during transport or storage (Zhivondov et al., 2003). Fruit strength was defined as the amount of healthy fruit left over during storage. Fungal diseases (*Botrytis cinerea*, *Rhizopus stolonifer*, *Colletotrichum gloeosporioides* and *Alternaria alternata*) are one of the main visible causes of loss of production during storage of fresh fruit (Bautista-Baños et al., 2003). Production rot processes can be reduced to some extent by minimizing mechanical damage during harvesting. The use of varieties with natural resistance to certain diseases, as well as the storage of products under optimal conditions, contributes to keeping the fruit for fresh consumption for a longer time. As a natural polymer with pronounced bacteriostatic and antifungal properties, chitosan limits diseases that occur in orchards and those that result from the storage of fresh fruit (Li & Yu, 2001).

In this study, the Regina variety and the Elite 17-37 Tsvetina hybrid were used. Pure chitosan, chitosan in combination with calcium lactate and chitosan in combination with alginate were applied for packaging.

2. Materials and methods

2.1. Materials

In the present study, a traditional cv. Regina and a new Elite 17-37 cv. Tsvetina hybrid were treated (Figure 1). The fruits were obtained in optimal maturated stage, from the Fruit Growing Institute, Plovdiv, Bulgaria.

(**a**) cv 'Regina' (**b**) cv. Tsvetina

Figure 1. Studied sweat cherry cultivars

Low molecular weight water-soluble chitosan (deacetylation rate: ≥90%, molecular weight 1.6 kD and viscosity 100 mPa.s) was used for edible coatings. Chitosan was purchased from Lyphar Biotech Co., LTD, China. The food grade Ca-lactate and the sodium alginate was bought from Sigma Aldrich, Bulgaria.

2.2. Sample preparation

Three types of edible coatings based on water-soluble chitosan were used in the experimental series: Pure chitosan solution containing 1% water-soluble chitosan and distilled water. A multicomponent system containing 1% chitosan and 1% Ca lactate. The coating is obtained by immersing the fruit once in the multicomponent solution. As a result, a single-layer coating is formed on the fruit. Two-layers coating of polyelectrolyte complex of 1% chitosan and 1% alginate. The coating is obtained by sequential immersion in solutions of chitosan and alginate. As a result, a layer of chitosan is first formed on the fruit, and then a second layer of alginate. After treatment, the experimental series of cherries were stored in a refrigerator at 4 °C for 3 weeks. The treated and untreated (control) fruits were analyzed during storage according to their physical, physicochemical, microbiological and sensory parameters. The end of storage was determined according to the number of remaining healthy samples.

2.3. Physical parameters

Quantitative changes (rate of non-decayed or browned fruits, weight and volume loss), and refractometric dry matter (Brix and refractive index) were reported on the first day and then once a week. The firmness of healthy fruits was determined by puncture test, on the same days as the other physical parameters.

2.4. Physicochemical parameters

These parameters were tested three times (1st 11th and 21st days) during the period for fruit pulp prepared from healthy samples.

2.4.1. The color of the fruit pulps

The color of the fruit pulp was detected by a "Colorgard 2000" colorimeter. The parameters are reported according to the CIE Lab system, where: L - illumination (L = 0 - black, L = 100 - white), + a - red color, -a - green color, + b - yellow color, -b - blue color (ASTM D2244-16, 2016). The color coordinates of each sample are the arithmetic mean of several measured coordinates. Saturation and color tone are the parameters that characterize the quality of color in the so-called physiological visual system and are related to the visual perception of color.

The color saturation C is calculated by the formula:

$$C = \sqrt{a^2 + b^2} \qquad (1)$$

The hue angle (h° value) shows the change in the color of fruit pulp, with a lower h° indicating more severe browning.

$$h = arctan\left(\frac{b}{a}\right) \qquad (2)$$

The color differences between the individual samples are determined by the values ΔL, Δa and Δb, and ΔE is a generalized parameter of the final color difference. The difference in color change ΔE is determined by the formula (Atarés & Chiralt, 2016):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \qquad (3)$$

Where:

$\Delta L = L_i - L_0$

$\Delta a = a_i - a_0$

$\Delta b = b_i - b_0$

"0" - etalon

"i" – probe

The parameter ΔE can be used to determine the change of fruit color (browning) during the storage time, as L_0, a_0, b_0 are the coordinates of fresh fruits L_i, a_i, b_i are the coordinates of stored fruits. The browning index (BI) used as a parameter of brown intensity is calculated by the following formula (Kumar et al., 2018):

$$BI = [100 \cdot (x - 0.31)]/0.172 \qquad (4)$$

Where

$$x = \frac{(a + 1.75 \cdot L)}{(5.646 \cdot L + a - 3.012 \cdot b)} \qquad (5)$$

2.4.2. Anti-oxidant activity

One of the methods for determining antioxidant activity is the DPPH test (Arnao et al., 2001). The method is based on measuring the decrease in the concentration of free radicals under the influence of the test sample. 2,2-diphenyl-1-picrylhydrazyl (DPPH) is used as a source of free radicals. Measurement of the decrease in concentration is performed by determining the decrease in specific absorption at a wavelength of 515 nm. The results of the analysis can be presented as a percentage decrease in the concentration of free radicals in a solution containing the measured sample compared to a solution with the same amount of distilled water added. If a comparison is sought not only in one series but between different series or different methods of determination, the results are presented as trolox equivalents (TE), the reaction system being calibrated by adding exact amounts of the standard antioxidant Trolox and the results are presented in units of

TE (trolox equivalent). An UV/VIS spectrometer "Thermo EVOLUTION 201" was used in these experiments

2.4.3. Active acidity (pH)

The pH values of the cherries were detected using a pH meter (Milwaukee Model MW1 02-FOOD, USA), with temperature compensation.

2.4.4. Determination of titratable acidity

Titratable acidity was determined as described by Zhang et al. (2016). The titratable acidity is expressed as the percentage of malic acid (%).

2.5. Microbiological parameters

In parallel with the physicochemical tests on microbiological criteria for foodstuffs, according to COMMISSION REGULATION (EC) № 2073/2005 of 15 November 2005 (EFSA 2010), the total microbial count (BS EN ISO 4833-1: 2013), the total amount of molds and yeasts (BS ISO 21527-2: 2011), Enterobacteriaceae (BS ISO 21528-2: 2017), Escherichia coli (BS ISO 16649-2: 2014), Sulfite reducing and clostridia (BS EN 26461-2: 2004) were determined for one third of the healthy samples.

2.6. Sensory analysis

Consumer test, with a 9-point scale for quality assessment was done. The whole cherry fruits were evaluated by thirty volunteers between 20–50 years old, who liked and eat cherries frequently. A total of 12 samples from 4 varieties coated or uncoated, labelled with 3 digits numbers were randomly provided to the panellists. The evaluated samples for each session comprised four samples, one coated with Chitosan (1%), one with Chitosan (1%) & Calcium Lactate (1%) and one with Chitosan (1%) & Alginate (1%), and non-coated control. A glass containing potable water and pieces of non-salted cracker were provided to panellists for eliminating the residual taste between samples. Appearance, shape&size, color, fruit taste, aroma, firmness and browning around the stone were the evaluated attributes by the panellists. Each attribute was scored on a structured 9 points scale for quality evaluation labelled from “absolutely no quality” (1) to “extremely good quality” (9) (Wichchukit & O'Mahony, 2015).

2.7. Statistical analysis

Statistical analysis was performed using the statistical package Statistica. A multifactor ANOVA with posterior Multiple Range Test was used to find significant differences ($p=0.05$) among variety, storage time and edible coating on the sensory evaluation profile and in the analysis of the data from the other experiments as well.

3. Results and discussions

3.1. Quantitative changes

Relative number of healthy fruits: The percentage of healthy fruit changes during the full storage time. The fastest changes are observed in non-packaged fruit (to 35-40%). In both cultivars, the smallest loss can be reported when packed with chitosan (to ~77 %). The effect of packages with chitosan and calcium lactate and chitosan and alginate is almost the same (to 65-70%).

Weight and volume losses are expressed as the ratio between the weight or volume of the current day of storage and the first day (100%). As a result of volume losses, the fruits wrinkle. The losses are greatest in the control samples. The smallest volume losses were found in the cv. Tsvetina, packed with chitosan alginate (to about 73.6%), followed by a package of pure chitosan by 71.5%, a package of chitosan and calcium lactate by 70% and the control by 64%. In the cv. Regina, the losses are smaller. The lowest loss was observed in chitosan-calcium lactate packaging to 82%, followed by chitosan-packing 79%, chitosan-alginate packaging 73.5% and control by 72%. Weight loss is associated with water loss (dehydration) and loss of respiration. The differences in weight loss are smaller. For the cv. Tsvetina the weight loss of the packaged fruits is to about 77-78%, and for the control - to 70%. For the cv. Regina, the loss in fruits packed with chitosan and chitosan calcium lactate is to 83-85%, for fruits packed in chitosan alginate is 80% and for non-packed fruits is 76%.

3.2. Refractometric dry matter content

During the storage time for all tested samples (packaged and unpackaged) the refractive index and the refractometric dry matter content (Brix) are constantly increasing, and the growth of the change is different depending on the composition of the package and the cultivar. These changes are the result of drying (weight and volume losses). The increase may be due to the breakdown of starch to sugars, to a decrease in respiration rate and the conversion of sugars to CO_2 and H_2O (Ghasemnezhad et al., 2011), to the hydrolysis of cell wall polysaccharides (Comabella & Lara, 2013) and to an increase of the dry content due to water loss (Petriccione et al., 2015).

The fastest changes are in the control samples (Table 1).

Table 1. Refractive index and refractometric dry content content (Brix) of sweet cherries

Cult.	Treat-ment	Day	Refractive index	Brix	Cult.	Refractive index	Brix
Tsvetina	Control.	1	1.3656±0.0021^{a}	21.26±1.29^{a}	Regina	1.3587±0.0044^{a}	16.80±2.86^{a}
		8	1.3686±0.0022^{b}	22.81±1.30^{ab}		1.3596±0.0026^{ab}	17.36±1.60^{ab}
		15	1.3748±0.0021^{c}	26.26±1.29^{c}		1.3646±0.0020^{c}	20.37±1.22^{c}
		21	1.3794±0.0028^{cd}	28.73±1.27^{d}		1.3653±0.0035^{cd}	20.92±1.92^{cd}
	Ch.	1	1.3653±0.0029^{a}	20.81±1.77^{a}		1.3586±0.0026^{a}	16.76±1.57^{a}
		8	1.3653±0.0025^{a}	20.85±1.47^{a}		1.3593±0.0035^{ab}	17.18±2.10^{ab}
		15	1.3685±0.0027^{b}	22.71±1.54^{b}		1.3624±0.0021^{c}	19.10±1.32^{c}
		21	1.3705±0.0050^{bc}	23.86±2.86^{bc}		1.3662±0.0049^{d}	19.36±2.92^{d}
	Ch+CaL	1	1.3632±0.0027^{a}	19.56±1.50^{a}		1.3579±0.0041^{a}	16.36±2.49^{b}
		8	1.3667±0.0032^{b}	21.56±1.87^{b}		1.3623±0.0058^{b}	18.94±3.64^{a}
		15	1.3704±0.0022^{bc}	24.01±1.30^{c}		1.3636±0.0014^{bc}	19.75±0.81^{bc}
		21	1.3828±0.0039^{c}	25.82±2.29^{c}		1.3632±0.0030^{bc}	19.52±1.80^{bc}
	Ch+Alg	1	1.3638±0.0034^{a}	19.93±2.02^{a}		1.3561±0.0029^{a}	15.26±1.73^{a}
		8	1.3653±0.0038^{ab}	20.78±2.24^{ab}		1.3560±0.0032^{a}	16.10±2.02^{a}
		15	1.3658±0.0037^{ab}	21.06±2.20^{bc}		1.3618±0.0032^{b}	18.73±1.99^{b}
		21	1.3667±0.0012^{b}	21.74±0.75^{c}		1.3679±0.0058^{c}	19.33±3.45^{c}

$^{a, b, c, d}$: the different letters mean a significant difference between the values. (p=0.05)

In confirmation of expectations, the packaging reduces the rate of change and preserves the hydration of the fruit. This result is due to the ability of edible packaging to modify the internal atmosphere (Martínez-Romero et al., 2006). The slowest increase is observed for the cv. Tsvetina, packed with chitosan-alginate, where the studied parameters are almost constant. The weakest is the effect of packaging in the cv. Tsvetina, packaged in a multi-component coating of chitosan and calcium lactate. The increase in performance is slower in the cv. Regina, most likely because this cultivar contains less dry content at the beginning of storage. Cv. Regina with chitosan packaging responds slowest in dry content increasing. The results are consistent with published literature data (Mali & Grossmann, 2003; Serrano et al., 2005).

3.3. Texture changes

Sweet cherries are crunchy fruits. When analyzing the texture of crunchy fruit, the most appropriate to use is a destructive test. The parameters of the puncture test are shown in Table 2. From the parameters determined in this test the reducing of the yield stress indicates the softening of the fruit. The rate of change in texture depends on the variety and the packaging. Non-coated fruit (control) changes the fastest. The slowest change in the cv. Tsvetina is observed in the package chitosan-calcium lactate, and in this cultivar the change is the smallest in the package chitosan-alginate.

Table 2. Texture parameters of the sweet cherries during the storage period

Cult.	Treat.	Day	Yield point				Rupture point		
			Stress, kPa	Relative deformation	Young modulus, kPa	Deformation work, kPa	Stress, kPa	Relative deformation	Deformation work, kPa
Tsvetina	Cont.	1	286.8±40.8	0.164±0.029	1324.0±223.0	16.8±3.4	378.4± 75.1	0.290±0.024	71.0± 9.3
		7	221.4±56.0	0.215±0.052	1192.7±249.2	27.1±5.2	587.5±115.0	0.340±0.070	95.1±10.7
		14	211.7±32.7	0.230±0.040	1097.3±201.9	15.4±1.6	387.4± 66.9	0.436±0.086	67.9± 6.9
		21	161.3±26.1	0.247±0.016	753.7±157.1	14.5±1.9	569.6±136.8	0.439±0.057	81.8±10.0
	Ch.	1	290.7+40.6	0.158±0.026	1392.8±246.3	16.2±3.2	360.0± 63.5	0.273±0.048	50.6± 6.8
		7	277.8+68.4	0.244±0.053	1346.3±236.4	31.0±6.0	742.3±146.6	0.398±0.079	87.0±13.8
		14	257.8+42.3	0.280±0.053	1130.7±198.9	19.1±3.3	580.2±102.9	0.434±0.081	86.8±11.1
		21	217.4+12.5	0.296±0.052	859.1±126.5	18.7±3.0	602.4± 77.8	0.452±0.042	70.9± 7.2
	Ch+CaL	1	279.1±50.1	0.159±0.030	1604.5±227.3	20.9±3.8	409.4± 51.8	0.280±0.030	59.9± 6.3
		7	268.3±49.1	0.202±0.035	1404.7±247.1	19.1±3.7	631.1±121.4	0.360±0.066	65.6±10.8
		14	254.3±56.1	0.279±0.084	1227.4±290.9	24.3±3.5	631.8±108.6	0.417±0.063	84.1±13.4
		21	214.3±82.5	0.379±0.052	777.5±153.8	14.6±1.9	370.7± 71.2	0.439±0.076	61.4± 7.7
	Ch+Alg	1	295.7±39.8	0.155±0.019	1515.3±192.6	17.5±3.2	367.6± 51.1	0.263±0.032	51.4± 8.3
		7	252.8±54.0	0.224±0.042	1410.5±264.5	27.4±5.0	631.3±121.4	0.381±0.071	71.3±10.3
		14	236.5±48.5	0.239±0.063	1190.2±206.3	21.5±2.4	409.7± 35.5	0.413±0.077	61.7± 5.8
		21	184.1±28.1	0.283±0.041	757.0±124.8	18.2±3.7	445.1± 61.6	0.440±0.054	76.8± 8.8
Regina	Cont	1	176.2±21.9	0.123±0.026	972.5±172.7	7.1±1.5	565.0± 53.2	0.335±0.059	50.0± 6.5
		7	137.9±26.5	0.161±0.058	859.3±171.1	17.6±3.2	422.3± 62.0	0.429±0.055	47.5± 4.5
		14	133.2±31.9	0.163±0.033	785.3±208.8	10.5±1.7	415.7± 46.0	0.434±0.065	42.4± 4.9
		21	114.2±22.0	0.183±0.027	692.4± 96.3	8.1±1.5	274.7±104.9	0.469±0.025	87.9±10.2
	Ch.	1	176.1±16.3	0.164±0.039	905.3±165.8	9.8±2.2	406.7± 53.6	0.361±0.077	45.7± 6.4
		7	149.6±24.4	0.175±0.024	893.1±180.5	6.9±1.2	347.1± 66.4	0.402±0.083	60.1±11.8
		14	135.3±29.1	0.196±0.039	853.2±130.9	10.4±2.2	329.2± 51.0	0.414±0.076	33.1± 3.2
		21	124.3±31.7	0.231±0.047	707.6±148.5	9.6±2.0	282.7± 67.3	0.465±0.067	61.3±11.0
	Ch+CaL	1	179.0±17.5	0.122±0.017	880.6±137.2	6.6±1.0	397.9± 57.4	0.363±0.072	50.3± 9.5
		7	150.1±20.4	0.133±0.023	876.0±153.5	7.6±1.4	373.2± 72.1	0.425±0.069	63.1±10.2
		14	113.8±28.4	0.157±0.033	819.6±150.2	8.7±1.8	367.2± 52.5	0.439±0.077	37.2± 4.4
		21	104.2±24.8	0.163±0.034	686.4±116.9	9.9±2.6	292.5± 86.8	0.454±0.056	63.1± 8.7
	Ch+Alg	1	176.1±23.0	0.114±0.024	1062.4±206.9	7.2±1.5	307.5± 60.5	0.389±0.076	56.2± 8.4
		7	145.4±24.8	0.120±0.025	978.9±175.1	6.0±1.2	352.0± 68.4	0.400±0.073	66.0±11.1
		14	127.8±25.5	0.161±0.030	821.6±157.9	5.6±0.5	316.6± 50.7	0.415±0.081	25.9± 3.7
		21	125.5±24.8	0.278±0.057	680.4±108.6	15.7±3.5	317.5± 63.3	0.485±0.079	74.5± 9.6

During the 3rd week, the hardness of all samples decreases at a different rate. The best preservation of the value of crunchiness is observed when applying a pure chitosan coating in both sweet cherry cultivars (figure 2).

The decrease in hardness can be explained by the delayed degradation of insoluble protopectins to the more soluble pectic acid and pectin.

During fruit ripening, depolymerization or shortening of the chain length of pectin substances occurs with an increase in pectinesterase and polygalactronase activity. Low oxygen concentrations and high carbon dioxide content reduce the activity of these enzymes and allow to reduce the hardness of fruits and vegetables during storage (Yaman & Bayoındırlı, 2002). The explanation for the increase in yield point deformation and rupture point deformation is similar.

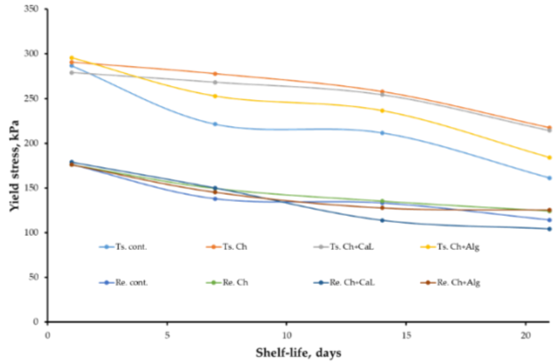

Figure 2. Changes in yield stress during storage of whole cherries with different packaging

The scientific literature reports that the softening process in cherries depends on the increase in the activity of polygalacturonase, β-galactosidase and pectin methyl esterase, which are responsible for the loss of fruit quality. The reduction in softening of coated samples can be explained by lower weight losses, reduced rot rate and less susceptibility to mechanical damage (Martínez-Romero et al., 2006).

3.4. Physicochemical parameters

The effect of chitosan packaging on antioxidant activity, active acidity (pH) and titratable acidity is shown in Table 3. According to our results, no change in in cherry acidity was observed during the storage (control and packaged cherries). Similar results have also been reported in the literature (Tokatlı, & Demirdöven, 2020). The antioxidant activity of sweet cherries decreases during storage. Its reduction depends on the cultivar of cherries and the composition of the package. For the cv. Tsvetina, the slowest is the reduction of AOA in the chitosan - calcium lactate package, faster in the chitosan-alginate packages and chitosan and the fastest without packaging. For the cv. Regina, the effect of packaging is reversed, the reduction rate is higher for packaged samples compared to the control. The overall reduction in antioxidant capacity (DPPH method) observed in all treatments can be explained by possible aging and breakdown, which often reduces antioxidant capacity (Kawhena et al., 2020).

Table 3. Physicochemical parameters during the storage of coated sweet cherries

Cult.	Treat.	Day	AOA, μmol/L	OTK%	pH	Cult.	AOA, μmol/L	Titratable acidity, %	pH
Tsvetina	Cont.	1	1288.68±12.50^{c}	0.61±0.012^{bc}	3.53±0.02^{a}	Regina	1265.72±30.15^{bc}	0.69±0.006^{bc}	3.25±0.02^{ab}
		11	1242.28±26.10^{ab}	0.60±0.012^{ab}	3.57±0.02^{b}		1242.88±40.70^{ab}	0.66±0.017^{ab}	3.22±0.02^{a}
		21	1212.23±10.06^{a}	0.58±0.012^{a}	3.59±0.02^{c}		1234.10±30.95^{a}	0.63±0.012^{a}	3.26±0.02^{b}
	Ch.	1	1282.42± 9.19^{c}	0.59±0.017^{ab}	3.55±0.03^{a}		1263.61± 8.51^{bc}	0.61±0.012^{b}	3.43±0.02^{a}
		11	1236.18± 5.94^{ab}	0.57±0.012^{ab}	3.59±0.01^{b}		1236.26±39.93^{ab}	0.59±0.006^{ab}	3.42±0.02^{a}
		21	1208.21±11.52^{a}	0.56±0.012^{a}	3.63±0.01^{c}		1212.60±28.54^{a}	0.58±0.012^{a}	3.44±0.02^{a}
	Ch+ CaL	1	1233.23±10.80^{bc}	0.76±0.006^{c}	3.52±0.03^{a}		1260.26±21.99^{c}	0.64±0.012^{bc}	3.34±0.02^{ab}
		11	1211.33± 8.78^{ab}	0.68±0.017^{b}	3.51±0.02^{a}		1222.16±39.35^{ab}	0.61±0.006^{bc}	3.32±0.02^{a}
		21	1193.57±11.13^{a}	0.64±0.012^{a}	3.58±0.01^{b}		1200.22±13.80^{a}	0.57±0.012^{a}	3.37±0.01^{b}
	Ch+Alg	1	1246.16± 9.07^{bc}	0.78±0.012^{c}	3.56±0.02^{ab}		1262.70±18.08^{c}	0.63±0.012^{ab}	3.32±0.02^{ab}
		11	1221.44±17.24^{ab}	0.65±0.006^{ab}	3.53±0.02^{a}		1224.44±35.82^{cb}	0.62±0.012^{ab}	3.31±0.02^{a}
		21	1205.14± 8.95^{a}	0.62±0.012^{a}	3.57±0.02^{b}		1206.09±10.67^{c}	0.59±0.012^{a}	3.35±0.02^{bc}

$^{a, b, c, d}$: the different letters mean a significant difference between the values. (p=0.05)

3.5. Colour parameters

The colour parametrs of the tested samples are presented in Table 4. Colour is an important factor for consumers when choosing food. Usually, the acceptability of processed fruits and vegetables increases when their colours are close to the original fresh and unprocessed. It is generally accepted that the most important parameter determining the acceptability of sweet cherries by consumers is the bright red colour (Crisosto et al., 2003). Changes in colour during ripening and storage of cherries are related to the content of anthocyanins (Serrano et al, 2005). The value of the illuminance parameter shows an increasing trend in the cv.

Tsvetina (with and without packaging), ie. the fruits become lighter, and only in the case of cherries covered with chitosan the values are approximately the same. The L parameter for the cv. Regina shows a decrease for all samples (the fruits become darker). The red colour index (a) increases during storage. This parameter shows the ripening of the fruit. The increase is less in packaged fruits. A smaller increase means a delay in over ripening and the intensity of respiration. A similar change in sensory quality was obtained by Martínez-Romero et al. (2006) and Tokatlı and Demirdöven (2020) for the packaging of cherries with aloe vera extracts and chitosan. The value of b becomes extremely high at the end of storage, decreasing only for cherries with a package of chitosan.

This indicator is probably related to the deterioration of the samples. Coated fruits show less and slower reduction in C than the control. The best preservation of the saturation value is observed with pure chitosan coating in both varieties of cherry.

Table 4. Colour parameters of the sweet cherries during the storage period.

Cult.	Treat.	Day	L	a	b	C	BI
Tsvetina	Cont.	1	15.02±0.98[a]	12.47±4.88[a]	0.57±0.10[ab]	12.48±4.88[a]	56.18+4.70[a]
		11	15.43±1.02[ab]	15.25±3.91[ab]	0.16±0.13[a]	15.25±3.91[ab]	62.16+8.86[ab]
		21	18.45±1.71[c]	23.58±0.68[b]	4.43±2.65[c]	24.08±1.12[c]	106.00+9.05[c]
	Ch.	1	15.01±1.17[a]	14.82±2.75[a]	0.11±0.08[a]	14.82±2.75[a]	61.97+8.53[a]
		11	15.15±0.54[ab]	14.85±2.40[a]	0.45±0.12[b]	14.86±2.40[a]	64.04+7.03[a]
		21	14.61±1.65[a]	16.29±2.95[ab]	0.57±0.09[b]	16.30±2.95[ab]	72.07+5.61[ab]
	Ch+CaL	1	15.40±2.19[a]	13.12±1.18[a]	0.16±0.04[a]	13.12±1.18[a]	55.29+8.60[a]
		11	14.91±1.96[a]	15.14±3.20[a]	0.79±0.79[ab]	15.17±3.24[ab]	68.11+9.26[ab]
		21	17.22±2.90[ab]	18.89±5.72[a]	2.96±1.60[c]	19.14±5.88[bc]	85.9+10.38[bc]
	Ch+Alg	1	16.01±0.98[a]	14.56±0.76[a]	0.94±0.07[a]	14.59±0.75[a]	63.95+1.96[a]
		11	16.56±0.84[ab]	14.95±2.11[a]	1.36±0.25[ab]	15.01±2.12[a]	68.08+6.44[a]
		21	18.78±0.77[c]	21.83±1.20[b]	3.35±0.26[c]	22.10±1.22[b]	92.34+6.95[b]
Regina	Cont.	1	15.83±0.41[b]	9.15±1.59[a]	0.30±0.03[a]	9.15±1.59[a]	40.61+6.52[a]
		11	12.48±3.21[a]	11.83±5.37[b]	1.40±0.20[b]	11.91±5.36[ab]	77.38+5.86[b]
		21	11.22±3.08[a]	13.91±3.84[b]	0.88±0.18[ab]	13.93±3.84[b]	87.95+8.91[bc]
	Ch.	1	14.36±3.22[b]	8.34±2.03[a]	0.68±0.26[ab]	8.36±2.04[a]	46.13+7.72[a]
		11	13.48±0.94[ab]	8.83±2.22[a]	0.53±0.13[ab]	8.85±2.22[a]	47.36+8.53[a]
		21	12.77±2.97[a]	9.46±3.97[a]	0.25±0.06[a]	9.46±3.97[ab]	52.77+7.62[ab]
	Ch+CaL	1	13.87±1.33[a]	6.81±0.93[a]	1.46±0.54[b]	6.98±1.00[a]	46.00+8.02[a]
		11	14.69±1.29[b]	10.21±4.48[ab]	1.10±0.39[ab]	10.27±4.49[ab]	53.74+9.04[ab]
		21	13.56±1.01[a]	11.47±3.31[b]	0.51±0.13[a]	11.49±3.30[b]	58.31+6.52[ab]
	Ch+Alg	1	15.49±1.62[b]	6.45±1.15[a]	1.44±0.31[b]	6.61±1.19[a]	39.74+8.26[a]
		11	15.15±2.46[b]	7.22±1.37[ab]	1.25±0.13[ab]	7.32±1.37[ab]	42.68+8.78[a]
		21	11.61±1.00[a]	8.47±0.39[bc]	0.71±0.21[a]	8.50±0.39[b]	54.29+7.54[ab]

[a, b, c, d]: the different letters mean a significant difference between the values during storage. ($p=0.05$)

The browning of the fruit increases during storage at most in the control samples (figure 3). Packaging reduces the rate of change. The increase is the smallest in cherries with chitosan packaging in both varieties.

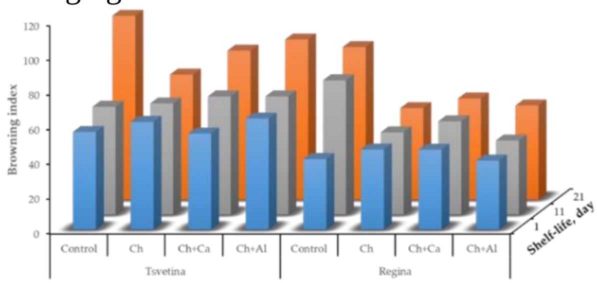

Figure 3. Browning index during storage of whole cherries with different packaging

3.6. Microbiological parameters

The samples remained safe without pathogens, but with increasing microbiological contamination (Table 5). Chitosan coating reduced the growth of fungi and other microbial contaminants on the surface of cherries for both varieties.

Antimicrobial effect of chitosan is due to the fact that the positively charged amino groups of chitosan bind to the negatively charged carboxyl groups on the bacterial cell membrane, thus changing the charge distribution on the cell surface, which leads to impaired membrane stability (Rabea et al., 2003; Dutta et al., 2009, Moreira et al., 2015).

Table 5. Microbiological parameters of the sweet cherries during the storage period

Cult.	Treat	Day	TMC	Molds and yeasts	Entero-bacteriaceae	Escherichia coli	Sulfitreducing and clostridia
			cfu/g	cfu/g	cfu/g	cfu/g	cfu/g
Tsvetina	Cont.	1	10800	4200	< 10	< 10	< 10
		11	78000	26500	< 10	< 10	< 10
		21	210000	34590	< 10	< 10	< 10
	Ch	1	10	< 10	< 10	< 10	< 10
		11	30	10	< 10	< 10	< 10
		21	830	80	< 10	< 10	< 10
	Ch+Ca	1	70	40	< 10	< 10	< 10
		11	1510	880	< 10	< 10	< 10
		21	4100	3700	< 10	< 10	< 10
	Ch+Al	1	1210	600	< 10	< 10	< 10
		11	6200	3100	< 10	< 10	< 10
		21	15000	7300	< 10	< 10	< 10
Regina	Cont.	1	3700	2680	< 10	< 10	< 10
		11	12500	5600	< 10	< 10	< 10
		21	59000	21400	< 10	< 10	< 10
	Ch	1	< 10	< 10	< 10	< 10	< 10
		11	10	< 10	< 10	< 10	< 10
		21	150	< 10	< 10	< 10	< 10
	Ch+Ca	1	300	120	< 10	< 10	< 10
		11	310	190	< 10	< 10	< 10
		21	5400	1600	< 10	< 10	< 10
	Ch+Al	1	1800	780	< 10	< 10	< 10
		11	7400	830	< 10	< 10	< 10
		21	16200	10400	< 10	< 10	< 10

The antimicrobial activity of multi-component packages is lower, but they also significantly reduce contamination compared to controls by the end of the storage period. A very similar result was reported in the Tokatlı and Demirdöven (2020) study for cherries.

3.7. The results of sensory analysis

On the first day, the points were approximately the same for packaged and unpackaged cherries in both varieties, only the chitosan-calcium lactate coated cherries received significantly smaller points (figure 4).

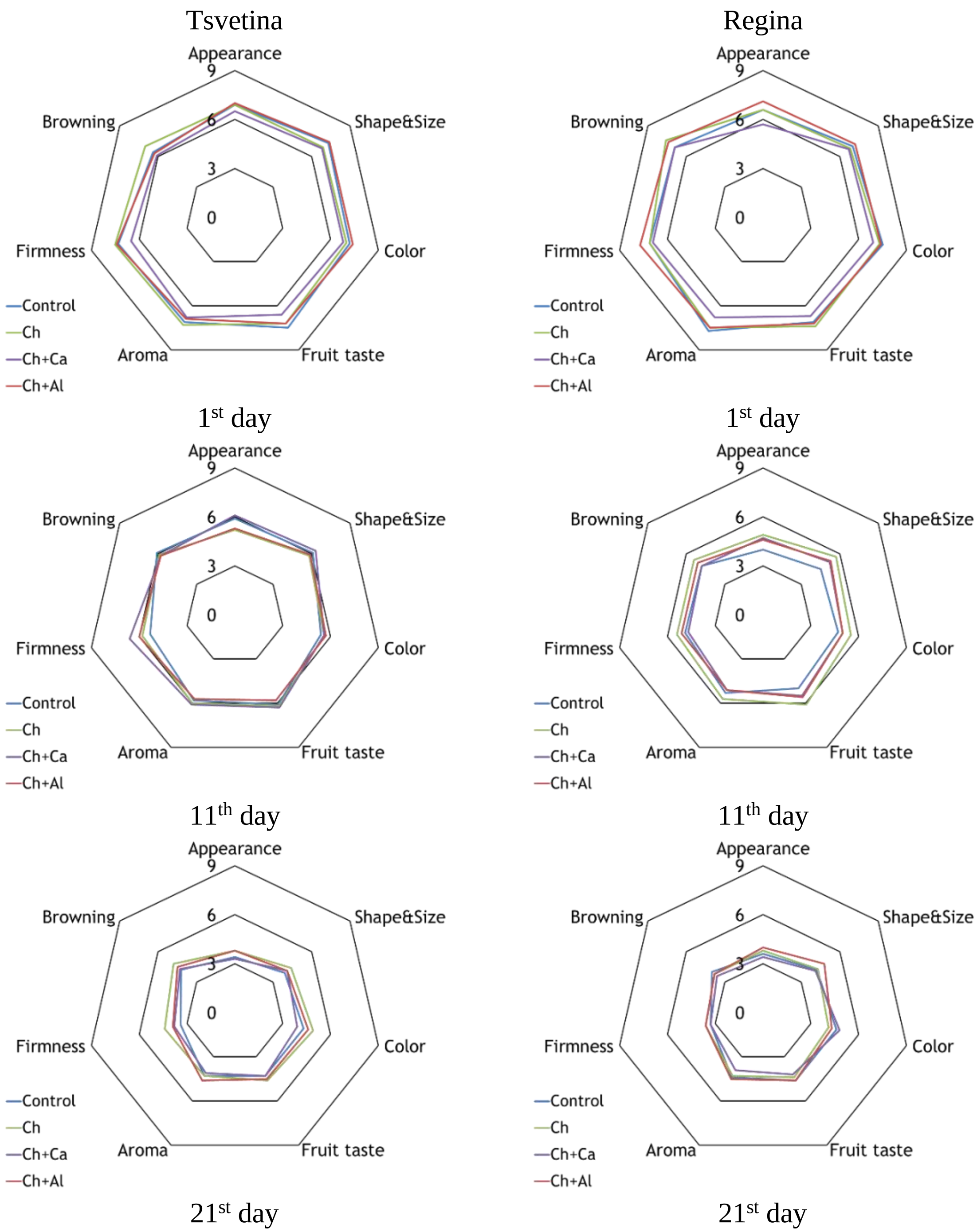

Figure 4. The results of the sensory analysis.

After 11 days of storage, the cv Tsvetina shows higher points compared to the cv Regina. On this day, the highest points are given to fruits packed with chitosan - calcium lactate.
The fruits with this packaging have retained their quality up to 90% of the original. The reason is the best preservation of the texture. The control and the other two packages show similar quality, about 80%. On the 11th day, the control has the least points in the Regina variety (66%). The fruits, coated in chitosan received the highest points (77%). Fruits packed with chitosan - calcium lactate and chitosan alginate received lower points (about 70%). At the end of storage, both varieties demonstrated low storage quality (55 to 60%). A similar change in sensory quality was obtained by Martínez-Romero et al. (2006) for the packaging of cherries with aloe vera extract.

4. Conclusions

Based on all the studied parameters, the Tsvetina variety retains better quality compared to the Regina variety.

Our experimental series provide information on the shelf life of coated cherries. It is no more than 21 days, about 3 weeks.

The samples remained safe without pathogens, but with increasing microbiological contamination. The smallest microbiological contamination is observed with pure chitosan coating. The other two coatings show a shorter extension of the storage period.

The pure chitosan coating showed the longest preservation of quality and safety between the applied treatments.

5. References

Aday, M. S., & Caner, C. (2010). Understanding the effects of various edible coatings on the storability of fresh cherry. *Packaging Technology and Science*, 23(8), 441-456. https://doi.org/10.1002/pts.910

Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food chemistry*, 73(2), 239-244. https://doi.org/10.1016/S0308-8146(00)00324-1

ASTM D2244-16 (2016), Standard Practice for Calculation of Color Tolerances and Color Differences from Instrumentally Measured Color Coordinates, *ASTM International*, *West Conshohocken*, PA, 2016, http://www.astm.org, DOI: 10.1520/D2244-16

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in food science & technology*, *48*, 51-62. https://doi.org/10.1016/j.tifs. 2015.12.001

Bautista-Baños, S., Hernández-López, M., Bosquez-Molina, E., & Wilson, C. L. (2003). Effects of chitosan and plant extracts on growth of Colletotrichum gloeosporioides, anthracnose levels and quality of papaya fruit. *Crop protection*, *22*(9), 1087-1092. https://doi.org/10.1016/S0261-2194(03)00117-0

Bernalte, M. J., Sabio, E., Hernandez, M. T., & Gervasini, C. (2003). Influence of storage delay on quality of 'Van'sweet cherry. *Postharvest Biology and Technology*, *28*(2), 303-312. https://doi.org/10.1016/S0925-5214(02)00194-1

BS EN 26461-2: 2004 (2004): Determination of sulfite reducing anaerobic bacteria (Clostridium perfringens) ISBN: 978 0 580 92184 1

BS EN ISO 21528-2:2017 (2017): Microbiology of the food chain. Horizontal method for the detection and enumeration of Enterobacteriaceae. Colony-count technique https://www.iso .org/standard/63504.html

BS EN ISO 4833-1: 2013 (2013): Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique (ISO 4833-1:2013) https:// www.iso.org/standard/53728.html

BS ISO 16649-2: 2001 (2001): Microbiology of Food and Animal Feeding Stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia Coli - Part 2: Colony-count technique at 44 °C Using 5-Bromo-4-Chloro-3-Indolyl

Beta-D-Glucuronide https://www.iso.org/standard/63504.html

BS ISO 21527-2: 2008 (2008): Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds Colony count technique in products with water activity less than or equal to 0,95 https://www.iso.org/standard/38276.html

Chaovanalikit, A., & Wrolstad, R. E. (2004). Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *Journal of food science*, 69(1). https://doi.org/10.1111/j.1365-2621.2004.tb17858.x

Comabella, E., & Lara Ayala, I. (2013). Cell wall disassembly and post-harvest deterioration of 'Sweetheart' sweet cherry fruit: involvement of enzymic and non-enzymic factors. *Pure and Applied Chemical Sciences*, 2013, vol. 1, núm. 1, p. 1-18. https://doi.org/10.12988/pacs.2013.321

Crisosto, C.H., Crisosto, G.M., Metheney, P., 2003. Consumer acceptance of 'Brooks' and 'Bing' cherries is mainly dependent on fruit SSC and visual skin color. *Postharvest Biol. Technol.* 28, 159–167. https://doi.org/10.1016/S0925-5214(02)00173-4

Dutta, P.K., Tripathi, S., Mehrotra, G.K., Dutta, J., 2009. Perspectives for chitosan based antimicrobial films in food applications. *Food Chem.* 114 (4), 1173–1182. https://doi.org/10.1016/j.foodchem.2008.11.047

EFSA (2010): European Food Safety Authority; The assessment of the comparison of the Australian monitoring programme for carcasses to requirements in Regulation (EC) No 2073/2005 on microbiological criteria on foodstuffs. *EFSA Journal* 2010; 8(3):1452. [51 pp.]. doi: 10.2903/j.efsa.2010.1452.

Ghasemnezhad, M., Nezhad, M. A., & Gerailoo, S. (2011). Changes in postharvest quality of loquat (Eriobotrya japonica) fruits influenced by chitosan. *Horticulture, Environment, and Biotechnology*, *52*(1), 40-45. https:// doi.org/10.1007/s13580-011-0028-5

Kawhena, T. G., Tsige, A. A., Opara, U. L., & Fawole, O. A. (2020). Application of Gum Arabic and Methyl Cellulose Coatings Enriched with Thyme Oil to Maintain Quality and Extend Shelf Life of "Acco" Pomegranate Arils. *Plants*, *9*(12), 1690. https://doi.org/10.3390/plants9121690

Kumar, P., Sethi, S., Sharma, R. R., Singh, S., & Varghese, E. (2018). Improving the shelf life of fresh-cut 'Royal Delicious' apple with edible coatings and anti-browning agents. *Journal of food science and technology*, *55*(9), 3767-3778. Doi: 10.1007/s13197-018-3308-6

Li, H., & Yu, T. (2001). Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. *Journal of the Science of Food and Agriculture*, *81*(2), 269-274. https://doi.org/10.1002/1097-0010(20010115)81:2<269::AID-JSFA806>3.0.CO;2-F

Malchev, S., & Zhivondov, A. (2016). Breeding programme for developing new sweet cherry cultivars in the Fruit Growing Institute, Plovdiv, Bulgaria. *Agricultural Science and Technology*, 8(4), 289-291. DOI: 10.15547/ast.2016.04.055

Mali, S., Grossmann, M.V.E., (2003). Effects of yam starch on storability and quality of fresh strawberries (Fragaria ananassa). *J. Agric. Food Chem.* 21, 7005–7011. https://doi.org/10.1021/jf034241c

Martínez-Romero, D., Alburquerque, N., Valverde, J. M., Guillén, F., Castillo, S., Valero, D., & Serrano, M. (2006). Postharvest sweet cherry quality and safety maintenance by Aloe vera treatment: a new edible coating. *Postharvest Biology and Technology*, *39*(1), 93-100. https://doi.org/10.1016/j.postharvbio.2005.09.006

Moreira, M. R., Cassani, L., Martín-Belloso, O., & Soliva-Fortuny, R. (2015). Effects of polysaccharide-based edible coatings enriched with dietary fiber on quality attributes of fresh-cut apples. *Journal of* *food science and technology*, *52*(12), 7795–7805. https://doi.org/10.1007 /s13197-015-1907-z

Petriccione, M., De Sanctis, F., Pasquariello, M. S., Mastrobuoni, F., Rega, P., Scortichini, M., & Mencarelli, F. (2015). The effect of chitosan coating on the quality and nutraceutical traits of sweet cherry during postharvest life. *Food and bioprocess technology*, 8(2), 394-408. https://doi.org/10.1007/s11947-014-1411-x

Rabea, E.I., Badawy, M.E.T., Stevens, C.V., Smagghe, G., Steurbaut, W., (2003). Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules*. 4 (6), 1457–1465. https://doi.org/10.1021/bm034130m

Schick, J. L., & Toivonen, P. M. (2002). Reflective tarps at harvest reduce stem browning and improve fruit quality of cherries during subsequent storage. *Postharvest Biology and Technology*, *25*(1), 117-121. https://doi.org/10.1016/S0925-5214(01)00145-4

Serrano, M., Martínez-Romero, D., Castillo, S., Guillén, F., Valero, D., (2005). The use of antiflingal compounds improves the beneficial effect of MAP in sweet cherry storage. *Inno. Food Sci. Emerg. Technol.* 6, 115–123. https://doi.org/10. 1016/j.ifset.2004.09.001

Tokatlı, K., & Demirdöven, A. (2020). Effects of chitosan edible film coatings on the physicochemical and microbiological qualities of sweet cherry (Prunus avium L.). *Scientia Horticulturae*, *259*, 108656. https:// doi.org/10.1016/j.scienta.2019.108656

Vargas, M., Chiralt, A., Albors, A., & González-Martínez, C. (2009). Effect of chitosan-based edible coatings applied by vacuum impregnation on quality preservation of fresh-cut carrot. *Postharvest Biology and Technology*, *51*(2), 263-271. https://doi.org/ 10.1016/j.postharvbio.2008.07.019

Wichchukit, S., & O'Mahony, M. (2015). The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives. *Journal of the Science of Food and Agriculture*, 95(11), 2167-2178. https://doi.org/10.1002/jsfa.6993

Yaman, Ö., & Bayoındırlı, L. (2002). Effects of an edible coating and cold storage on shelf-life and quality of cherries. *LWT-Food science and Technology*, *35*(2), 146-150. https://doi.org/10.1006/fstl.2001.0827

Zhang, L., Chen, F., Zhang ,P., Lai, S., Yang, H. (2017): Influence of rice bran wax coating on the physicochemical properties and pectin nanostructure of cherry tomatoes; *Food and Bioprocess Technology*, 10 (2017), pp. 349-357 DOI: https://doi.org/10.1007/s11947-016-1820-0

Zhivondov, A., Gercheva, P., & Djouvinov, V. (2003, September). Some results of a sweet cherry breeding programme in Bulgaria. In *XI Eucarpia Symposium on Fruit Breeding and Genetics 663* (pp. 739-742). DOI: 10.17660/ActaHortic.2004.663.133

Acknowledgment

This research was funded by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018" and by Agriculture Academy of Bulgaria 2019 “TN3: Using of natural components for preparing of functional foods (2019-2021)” WP: “Using of natural polymers, like edible coatings for extension of the fruit shelf-life”.