


# Analysis of chitosan treatment on white and black sweet cherry

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### ABSTRACT

Bicolor (Rosaline) and black (Regina) sweet cherry cultivars were treated with chitosan-Ca-lactate and chitosan-alginate solutions. The chitosan coating is biocompatible, nontoxic and possesses antimicrobial activity. The sample series (five replicates of thirty pieces from each variety and each treatment, and a control) were refrigerated at 4 °C for 21 and 28 d, to the end of shelf-life. Physical (visual sorting, weight loss and texture of intact fruit), physicochemical (TSS, antioxidant activity, and pH of the pulp), and microbiological properties (total number of microorganisms, *Escherichia coli*, fungi and yeasts) were investigated weekly. For the last week only the Regina cultivar had acceptable appearance, the other cultivar was discarded after 21 d. The chitosan-alginate treatment preserved the texture, showed smaller weight loss, higher antioxidant preservation and smaller microbial contamination than the samples with chitosan-Ca-lactate on both cultivars. Based on the results, the edible coating can help to preserve the nutritional value of fresh fruit and this technology can be useful in preparing the ready-to-eat fruit salads or in decoration of confectionery products.

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### KEYWORDS

shelf-life time, physical properties, edible coating, *Prunus avium*

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## INTRODUCTION

Fresh fruits are extremely perishable and more susceptible to postharvest spoilage due to high moisture content (80–90%) limiting the storage period and marketing life and causing high economic losses around the world (Mainsnam et al., 2017). The quality of fruits can be maintained but not improved after harvesting; therefore, it is essential to harvest fruits at proper stage and maturity. The edible coating can be one of the tools of sustainable food systems to maintain food quality and safety by reducing postharvest losses. Edible coatings are non-pollutant natural polymers thin wrapping layers on the surface of the food. They serve as a barrier between the food and the environment, during handling, transport and storage. They have functional and/or anti-microbiological effect (García et al., 2014). They are formed from three types of biological materials: hydrocolloids (polysaccharides and proteins), lipids and composite materials. There are different techniques for application of the edible coating on the fruit, such as brushing, dipping or spraying the coating solution on the food surface (Misir et al., 2014). The chitosan is a polysaccharide coating material, produced by deacetylation of chitin (obtained from shrimp, crab and crawfish shells, and mushroom waste). It has functional properties like antimicrobial activity, antioxidant activity, film forming ability, texturizing and binding property. It is one of the widely used coating materials (Lin et al., 2018), which delays ripening and color changing, and reduces ethylene production. Alginate is a water soluble, linear polysaccharide extracted from brown seaweed. Alginate has been reported to be mucoadhesive, biodegradable, and biocompatible gelling or thickening agent. Chitosan can interact ionically with several poly-anions, such as alginate (Bellich et al., 2016).

Sweet cherry (*Prunus avium* L.) is one of the most commercially important *Prunus* fruit tree species planted in temperate climate zones and in Bulgaria (Malchev & Zhivondov, 2016; Zhivondov et al., 2011) with high sensitivity for postharvest loss, short ripening and storage period. Sweet cherries are non-climacteric with high transpiration rate and a susceptibility to fungal rots and physiological disorders (Alique et al., 2005). The storage period can be extended with cooling because the sweet cherry is considered to be a non-chilling sensitive fruit (Petriccione et al., 2015).

‘Rosalina’ cultivar (2009) is one of the first bicolor sweet cherry cultivars from the selection program of Fruit Growing Institute (Plovdiv, Bulgaria). The cultivar ‘Rosalina’ possesses high and regular productivity and the fruit is resistant to cracking with very consistent pale, yellow mesocarp, uncolored juice and strong acidity (Zhivondov, 2011). ‘Regina’ is a high-quality, late-season cherry cultivar that exhibits excellent rain crack resistance. The fruit is very large and firm, with a mild, pleasant flavor (Long et al., 2007).

In this study, the effect of chitosan based edible coatings on the two above mentioned sweet cherry cultivars during storage was evaluated.

## MATERIALS AND METHODS

Fruit from Rosalina and Regina sweet cherry cultivars were harvested for shelf-life experiments from the Fruit Growing Institute – Plovdiv, Bulgaria. Those two varieties ripen more or less at the same time; they were harvested and treated on the same day. The food-grade, water-soluble chitosan (low molecular weight) 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.



## Treatments

All of the fruit were selected without injury and with stalk, were carefully washed and dried before the experiments. Three types of coating solutions were prepared: 1. chitosan (1%) -Ca-lactate (1%), 2. Chitosan (1%) and 3. Sodium-alginate (1%) solutions with distilled water. The fruit were immersed for 10 min. to chitosan-Ca-lactate (1%) solution (Ch-Ca – monolayer treat) or at first to chitosan (1%), after drying to alginate solution (1%) for bilayer treat (Ch-Al), and dried for 10 min. The samples were refrigerated at 4 °C on opened trays (30 pieces/tray). 10 trays for each coating variants and control were prepared from both varieties. The physical, physicochemical and microbiological parameters were investigated on one tray (extended to 30 fruits) from all series each week during the storage.

## Visual appearance loss

The damaged (injured, browned or rotted) pieces were selected and the quantity of them is expressed in % for the trays. All of the trays were allowed for selection at each time.

## Weight loss

The identified fruit were weighed at each experimental date before they were selected for investigation or wasted. The weight loss was calculated as the % of weight difference compared to the initial weight.

## Texture

Ten fruits from all selected trays were measured with a TAXT2i Texture Analyzer (Stable Micro Systems Ltd, Godalming, UK) using puncture test with cylindrical probe ( $d = 5$  mm, deformation speed = 1 mm/s, max. deformation = 8 mm, [Aday and Caner, 2010](#)).

The freshness of the sweet cherry characterized by the crunchiness (hard peel and flesh):

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

where  $F_f$  = yield force,  $F_r$  = rupture force,  $\ell_f$  = yield deformation,  $\ell_r$  = rupture deformation.

Third of the fruit from a tray was pitted and meshed to pulp, together with the peel but without the stalk for physicochemical tests and another third for microbiological tests.

*Soluble solid content* (TSS, °Brix) was measured by ABBE type refractometer at 20 °C.

*pH of the pulp* was measured by an INOLAB pH 7110 type (RADELKIS, Hungary) pH meter at 20 °C in five repetitions. The instrument was calibrated at pH 4.0 and 7.0.

## Antioxidant activity

Total antioxidant activity (TAA) was quantified by the method based on the capacity of different components to scavenge the DPPH radical cation compared to the standard antioxidants (ascorbic acid and Trolox) in a dose response curve. The absorbance at 515 nm of the extract was measured by spectrophotometer (UVVIS EVOLUTION 201 Thermo Scientific USA). The results are expressed as Trolox equivalent mg/100 g ([Arnao et al., 2001](#)).

The *total number of microorganisms* (TNM – EN ISO 4833-2:2013), the *total coliform bacteria* (ISO 16649-2:2001) and the *total yeasts and molds* (TYM – EN ISO 21527-2:2011) were



measured based on the plate counting method. The results were expressed as a logarithm of colony forming units ( $\log_{10}$  cfu/g).

The received data was statistically evaluated for the differences among treatments and storage days by one way ANOVA method. Homogeneity groups of the samples were analyzed based on the significance level of the differences by post hoc Fisher (LSD) test.

## RESULTS AND DISCUSSIONS

### Visual appearance loss

During the storage, the quantity of healthy fruit decreased. That decreasing depends on both the cultivar and the coating. From the cultivar, 'Rozalina' there were not enough healthy fruit left for further experiments after the 22nd d. The cultivar 'Regina' could be stored for 29 d. To the end of the shelf life time, the appearance of the fruits also changed. Most of the control fruit became wrinkled during 29<sup>th</sup> d. The treated samples better tolerate the cold storage. The loss of samples among the Ch-Al coated fruit was lower than among the Ch-Ca coated samples (Fig. 1).

The *weight-loss* is mainly due to water-loss caused by transpiration and respiration. The sweet cherry has low skin diffusion resistance (Serrano et al., 2005) and high surface/volume ratio (Conte et al., 2009; Wani et al., 2014). During cold storage, coated samples show lower weight loss, because they have lower respiration rates (Bautista-Banos et al., 2006). The delay in weight loss with Ch-Al coating was longer than with the Ch-Ca.

### Texture changes

The fresh sweet cherry fruit have high crunchiness (hard peel and flesh). The crunchiness of the cv. 'Rozalina' was higher during the storage time than that of the cv. 'Regina' (Fig. 2). The Ch-Al treated samples showed higher crunchiness because the ionic complex made the peel stronger and the flesh harder, with high force ionic bindings (Diaz-Mula et al., 2012).

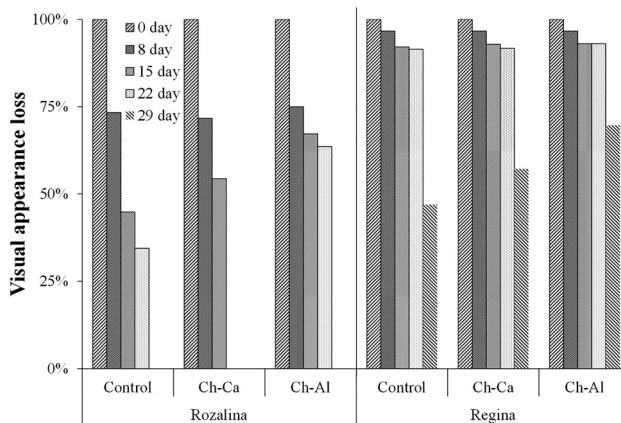


Fig. 1. Visual appearance loss



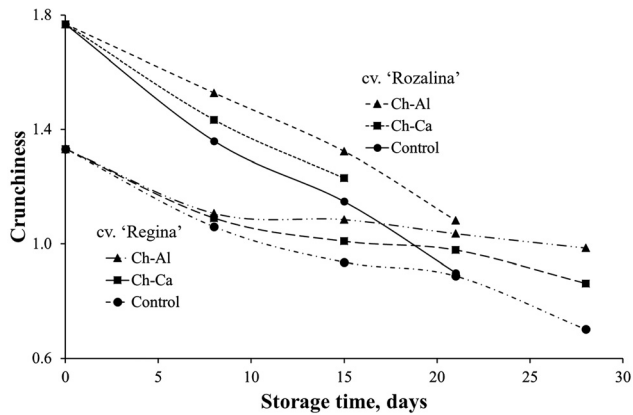


Fig. 2. Crunchiness

### Soluble solid content

The TSS values of the coated and uncoated fruit increased over the 22 d or 29 d of cold storage period (Table 1). The increase could be attributed to the breakdown of starch to sugar, to the decrease in respiration rate and conversion of sugars into  $\text{CO}_2$  and  $\text{H}_2\text{O}$  (Ghasemnezhad et al., 2011), to the hydrolysis of cell wall polysaccharides (Comabella and Lara, 2013), and to the increase of dry matter due to water loss (Petriccione et al., 2015). The changes are smaller in the coated samples, because the coatings modified the internal atmosphere, reduced the respiration activity and the water-loss (Dong et al., 2004; Zsom et al., 2016). According to our results, the Ch-Ca coating is slightly better in the preservation of the TSS than the Ch-Al treatment.

### pH

The chitosan based coatings reduced pH increasing significantly during the shelf life (Table 1). The higher acidity loss in uncoated fruit could be explained by respiratory metabolism (Diaz-Mula et al., 2012). The acidity loss was smaller with bilayer Ch-Al coating maybe due to the smaller weight-loss. Lower acidity loss with chitosan and/or alginate based coatings are reported for different fruits in the literature as well (strawberry, guava, and litchi by Dong et al., 2004; Hernandez-Munoz et al., 2008; Hong et al., 2012).

### Antioxidant activity

The sweet cherry is a very good source of natural antioxidants (Ferretti et al., 2010). Chitosan based coatings delay the fruit senescence that is associated to enzymatic and non-enzymatic antioxidant systems (Usenik et al., 2008). The antioxidant activity is much higher for the 'Regina', but the decrease is smaller for the 'Rozalina' (Table 1). The shown cultivar dependence is known from other studies as well (Pasquariello et al., 2015). The results obtained show how the coatings delay the decreasing of the antioxidant activity. The reducing effect of the Ch-Al bilayer coatings is higher than the Ch-Ca.



Table 1. Result of physico-chemical and microbiological properties

| Cultivar   | Treat    | d*                         | TSS, °Brix                     | TAA, mg/100 g                  | pH                             | TNM, log <sub>10</sub> cfu/g | TYM log <sub>10</sub> cfu/g |                             |
|------------|----------|----------------------------|--------------------------------|--------------------------------|--------------------------------|------------------------------|-----------------------------|-----------------------------|
| 'Rozalina' | Cont.    | 0                          | 15.6 ± 0.10 <sup>a</sup>       | 2,207.10 ± 0.42 <sup>d</sup>   | 3.68 ± 0.03 <sup>a</sup>       | 4.67 ± 0.35 <sup>a</sup>     | 3.22 ± 0.15 <sup>a</sup>    |                             |
|            | Cont.    | 8                          | 16.0 ± 0.05 <sup>b,y</sup>     | 2,170.88 ± 0.33 <sup>c,z</sup> | 3.92 ± 0.03 <sup>b,z</sup>     | 5.22 ± 0.48 <sup>ab,y</sup>  | 3.50 ± 0.18 <sup>ab,z</sup> |                             |
|            | Cont.    | 15                         | 17.5 ± 0.05 <sup>c,y</sup>     | 2,121.76 ± 0.44 <sup>b,z</sup> | 4.02 ± 0.04 <sup>c,y</sup>     | 5.47 ± 0.46 <sup>b,z</sup>   | 4.16 ± 0.23 <sup>b,y</sup>  |                             |
|            | Cont.    | 22                         | 17.7 ± 0.05 <sup>cd,y</sup>    | 2,040.88 ± 0.55 <sup>a,z</sup> | 4.08 ± 0.02 <sup>c,y</sup>     | 5.99 ± 0.61 <sup>bc,z</sup>  | 4.50 ± 0.24 <sup>bc,y</sup> |                             |
|            | Ch-Al    | 0                          | 15.6 ± 0.10 <sup>a</sup>       | 2,207.10 ± 0.42 <sup>d</sup>   | 3.68 ± 0.03 <sup>a</sup>       | 4.67 ± 0.35 <sup>a</sup>     | 3.22 ± 0.15 <sup>a</sup>    |                             |
|            | Ch-Al    | 8                          | 15.5 ± 0.10 <sup>a,z</sup>     | 2,200.59 ± 0.40 <sup>c,x</sup> | 3.99 ± 0.02 <sup>b,y</sup>     | 4.98 ± 0.26 <sup>a,z</sup>   | 3.38 ± 0.23 <sup>a,z</sup>  |                             |
|            | Ch-Al    | 15                         | 16.5 ± 0.05 <sup>b,z</sup>     | 2,181.18 ± 0.30 <sup>b,z</sup> | 3.96 ± 0.01 <sup>b,zy</sup>    | 5.15 ± 0.26 <sup>ab,z</sup>  | 3.50 ± 0.26 <sup>ab,z</sup> |                             |
|            | Ch-Al    | 22                         | 16.8 ± 0.10 <sup>bc,z</sup>    | 2,101.31 ± 0.48 <sup>a,y</sup> | 3.99 ± 0.02 <sup>b,z</sup>     | 5.29 ± 0.31 <sup>b,z</sup>   | 4.01 ± 0.19 <sup>b,z</sup>  |                             |
|            | Ch-Ca    | 0                          | 15.6 ± 0.10 <sup>a</sup>       | 2,207.10 ± 0.42 <sup>c</sup>   | 3.68 ± 0.03 <sup>a</sup>       | 4.67 ± 0.35 <sup>a</sup>     | 3.22 ± 0.15 <sup>a</sup>    |                             |
|            | Ch-Ca    | 8                          | 16.3 ± 0.10 <sup>b,x</sup>     | 2,194.65 ± 0.28 <sup>b,y</sup> | 3.90 ± 0.02 <sup>b,z</sup>     | 4.99 ± 0.27 <sup>ab,z</sup>  | 3.43 ± 0.21 <sup>a,z</sup>  |                             |
|            | Ch-Ca    | 15                         | 16.5 ± 0.10 <sup>bc,x</sup>    | 2,146.14 ± 0.19 <sup>a,y</sup> | 3.93 ± 0.02 <sup>b,z</sup>     | 5.37 ± 0.35 <sup>b,z</sup>   | 3.98 ± 0.27 <sup>b,y</sup>  |                             |
|            | 'Regina' | Cont.                      | 0                              | 17.2 ± 0.05 <sup>a</sup>       | 2,930.46 ± 0.40 <sup>e</sup>   | 3.61 ± 0.02 <sup>a</sup>     | 4.83 ± 0.22 <sup>a</sup>    | 3.17 ± 0.17 <sup>a</sup>    |
|            |          | Cont.                      | 8                              | 18.8 ± 0.05 <sup>bc,x</sup>    | 2,808.49 ± 1.19 <sup>d,z</sup> | 3.65 ± 0.04 <sup>a,z</sup>   | 4.93 ± 0.2 <sup>a,z</sup>   | 3.53 ± 0.17 <sup>ab,z</sup> |
|            |          | Cont.                      | 15                             | 18.5 ± 0.10 <sup>b,x</sup>     | 2,718.82 ± 0.55 <sup>c,z</sup> | 3.74 ± 0.03 <sup>b,z</sup>   | 4.99 ± 0.08 <sup>ab,z</sup> | 3.84 ± 0.18 <sup>b,z</sup>  |
| Cont.      |          | 22                         | 19.3 ± 0.10 <sup>d,x</sup>     | 2,637.73 ± 1.50 <sup>b,z</sup> | 3.93 ± 0.02 <sup>c,y</sup>     | 5.29 ± 0.12 <sup>b,y</sup>   | 4.32 ± 0.16 <sup>c,zy</sup> |                             |
| Cont.      |          | 28                         | 21.2 ± 0.05 <sup>c,x</sup>     | 2,488.46 ± 0.42 <sup>a,z</sup> | 3.99 ± 0.02 <sup>c,y</sup>     | 5.51 ± 0.34 <sup>bc,y</sup>  | 4.55 ± 0.21 <sup>c,z</sup>  |                             |
| Ch-Al      |          | 0                          | 17.2 ± 0.05 <sup>a</sup>       | 2,930.46 ± 0.40 <sup>e</sup>   | 3.61 ± 0.02 <sup>a</sup>       | 4.83 ± 0.22 <sup>a</sup>     | 3.17 ± 0.17 <sup>a</sup>    |                             |
| Ch-Al      |          | 8                          | 17.5 ± 0.10 <sup>ab,y</sup>    | 2,866.06 ± 0.21 <sup>d,x</sup> | 3.63 ± 0.02 <sup>a,z</sup>     | 4.87 ± 0.17 <sup>a,z</sup>   | 3.36 ± 0.20 <sup>a,z</sup>  |                             |
| Ch-Al      |          | 15                         | 17.3 ± 0.05 <sup>ab,y</sup>    | 2,802.88 ± 0.25 <sup>c,x</sup> | 3.73 ± 0.02 <sup>b,z</sup>     | 4.92 ± 0.31 <sup>a,z</sup>   | 3.76 ± 0.25 <sup>b,z</sup>  |                             |
| Ch-Al      |          | 22                         | 18.6 ± 0.05 <sup>b,y</sup>     | 2,776.46 ± 0.68 <sup>b,x</sup> | 3.77 ± 0.02 <sup>b,z</sup>     | 4.99 ± 0.13 <sup>ab,z</sup>  | 4.01 ± 0.37 <sup>bc,z</sup> |                             |
| Ch-Al      |          | 28                         | 19.0 ± 0.10 <sup>c,y</sup>     | 2,693.36 ± 0.60 <sup>a,x</sup> | 3.82 ± 0.02 <sup>bc,z</sup>    | 5.00 ± 0.24 <sup>ab,z</sup>  | 4.33 ± 0.38 <sup>bc,z</sup> |                             |
| Ch-Ca      |          | 0                          | 17.2 ± 0.05 <sup>b</sup>       | 2,930.46 ± 0.40 <sup>e</sup>   | 3.61 ± 0.02 <sup>a</sup>       | 4.83 ± 0.22 <sup>a</sup>     | 3.17 ± 0.17 <sup>a</sup>    |                             |
| Ch-Ca      |          | 8                          | 16.8 ± 0.05 <sup>a,z</sup>     | 2,828.24 ± 0.64 <sup>d,y</sup> | 3.67 ± 0.02 <sup>a,z</sup>     | 4.88 ± 0.18 <sup>a,z</sup>   | 3.46 ± 0.20 <sup>ab,z</sup> |                             |
| Ch-Ca      |          | 15                         | 16.8 ± 0.05 <sup>a,z</sup>     | 2,749.55 ± 0.42 <sup>c,y</sup> | 3.73 ± 0.02 <sup>ab,z</sup>    | 5.13 ± 0.15 <sup>b,z</sup>   | 3.80 ± 0.19 <sup>b,z</sup>  |                             |
| Ch-Ca      |          | 22                         | 17.5 ± 0.10 <sup>bc,z</sup>    | 2,648.28 ± 0.60 <sup>b,y</sup> | 3.79 ± 0.01 <sup>b,z</sup>     | 5.27 ± 0.14 <sup>b,y</sup>   | 4.20 ± 0.21 <sup>c,z</sup>  |                             |
| Ch-Ca      | 28       | 18.4 ± 0.10 <sup>c,z</sup> | 2,557.84 ± 0.95 <sup>a,y</sup> | 3.97 ± 0.02 <sup>c,y</sup>     | 5.33 ± 0.10 <sup>b,zy</sup>    | 4.51 ± 0.23 <sup>cd,z</sup>  |                             |                             |

abdce: post hoc Fisher (LSD) test for storage time; <sup>zyx</sup>: post hoc Fisher (LSD) test for threats.

\*Storage days.

## Antimicrobial activity of the coatings

The highest microbiological contamination was detected on the control samples (Table 1). Both chitosan-based coatings reduced the microbiological contaminations during the shelf-life period. The effect of the Ch-Al coating is higher, but the difference is not significant. From the viewpoint of the coliform bacteria all of the samples were safe during the full period.

## CONCLUSIONS

The chitosan based coatings preserved the sweet cherry quality during its shelf-life period. The Rozalina sweet cherry cultivar was more sensitive for the manipulation and had shorter storage time. Based on the quality and safety parameters the shelf life period for the cultivar 'Rozalina' was 21 d and for the cultivar 'Regina' was 28 d. The Ch-Al bilayer coatings preserved better the antioxidant activity, the crunchiness and the pH, then the Ch-Ca coating.



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