WHITE AND BLACK SWEET CHERRY CULTIVARS WITH CHITOSAN TREATMENT

Running title: Characterization of edible-coated 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 replicate thirty pieces from each variety and each treatment, and a control) were refrigerated at 4 °C for 21 and 28 days, up to the end of their shelf-life time. Physical (visual sorting, weight loss and texture of intact fruits), physicochemical (refractometrical dry content, antioxidant activity, and pH of the pulp), and microbiological properties (total number of microorganisms, *E. 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 days. The chitosan-alginate treatment preserved better the texture, showed smaller weight loss, higher antioxidant preservation and smaller microbial contamination on both cultivar. Based on the study, the edible coating can help to preserve the nutrition value of fresh fruit and this technology can be useful in preparing the ready-to-eat fruit salads or in decoration of confectionery products.

Key words: shelf life time, physical properties, edible coating.

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 (Maisnam 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 objectives 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 or 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

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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 material (Lin et al., 2018) which delays ripening and colour 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 agents. Chitosan can interact ionically with several polyanion, 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 (Zhivondov et al. 2003; Malchev & Zhivondov, 2016) with high sensitivity for postharvest loss, short ripening and storage period. Sweet cherries are a non-climacteric fruit with a high transpiration rate and a susceptibility to fungal rots and physiological disorders (Alique et al. 2005). The storage period can be extended with chilling because the sweet cherry is concerned like non-chilling sensitive fruit (Petriccione et al., 2015).

'Rosalina' cultivar (2009) is one of the first bicolor sweet cherry cultivar from the selection program of Fruit Growing Institute in Bulgaria. The cultivar 'Rosalina' possesses high and regular productivity and the fruits are resistant to cracking with very consistent pale yellow mesocarp, uncolored juice and strong acidity (Zhivondov, 2011). Regina is a high-quality, late-season cherry 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 is compared for the two above mentioned sweet cherry cultivars.

Materials and methods: Fruits from the two used sweet cherry cultivars were harvested for shelf-life experiments from the Fruit Growing Institute – Plovdiv. Those two varieties are ripened more or less in the same time; they were harvested and treated on the same day.

Treatments: Two type chitosan-based treatments and control samples were prepared. All of the fruits were selected without injuring and with stalk, carefully washed before the experiments. The fruits were immersed for 10 minutes to multicomponent chitosan-Ca-lactate (1%) solution (Ch-Ca) or at first to chitosan (1%), after drying to alginate solution (1%) for bilayer treat (Ch-Al), and dried for 10 minutes. 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 control samples and the other dried samples were sorted to opened trays (30 pieces/tray) and refrigerated at 4°C. The physical, physicochemical and microbiological parameters were investigated weekly on the fruits of one open tray.

Visual appearance loss: The non-intact (injured, browned or rotted) pieces were selected and the quantity of them is given in % for the trays. All of the trays were allowed for selection at each time.

Weight loss: the identified fruits were weighed up to they are selected for investigation or wasted. The weight loss was calculated like the % of original weight at the first time. After these methods, the selected tray was filled with some fruits from the other trays, to have 30 fruits on them.

Texture: 1/3 of the fruits from the tray (10 pieces) were measured with a TAXT2i Texture Analyzer (Stable Micro Systems Ltd, Godalming, UK) by puncture test with cylindrical probe (d=5 mm, deformation speed 1 mm/s, max. deformation up to 8 mm).

1/3 of the fruits from the tray was pitted and meshed to pulp, together with the peel but without the stalk for physicochemical and another 1/3 for microbiological tests.

Soluble solid content (TSS) was expected by ABBE type refractometer at 20 °C in five repetitions. The results are presented as percentage (°Brix).

Active acidity (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 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 absorbance of the extract was measured by spectrophotometer (UVVIS EVOLUTION 201 Thermo Scientific USA). The results are expressed as mg of 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 detected based on the horizontal method for enumeration. The results were expressed as a logarithm of colony forming units (lg cfu/g).

Results and discussions

Chitosan coatings form a semipermeable film that regulates gas exchange and reduces the transpiration rate, which is generally determined by the gradient of water vapor pressure between

the fruit and the surrounding air. These effects mitigate the quality and freshness reducing of the fruits during the chilled storage (Bautista-Banos et al. 2006).

Visual appearance loss: During the storage, the quantity of healthy fruits decreased. That decreasing depends on both the cultivar and the coating. From the cultivar 'Rozalina' not enough healthy fruits remind for further experiments after the 22nd day. The cultivar 'Regina' was rollout just on the 29th day. The end of the storage time in this cultivar was shown not just in the quantity of healthy pieces but also in the appearance of the pieces. Most of the fruits became wrinkled to the 29th day. The treated samples bear better the child storage. The Ch-Al coated fruits had more healthy fruits than the Ch-Ca coated samples (fig. 1).

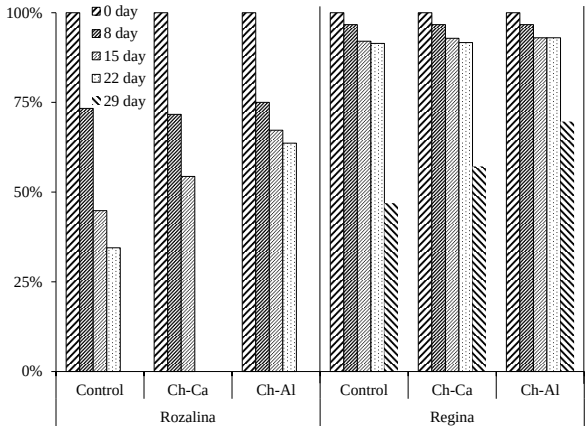

Figure 1 Visual appearance loss

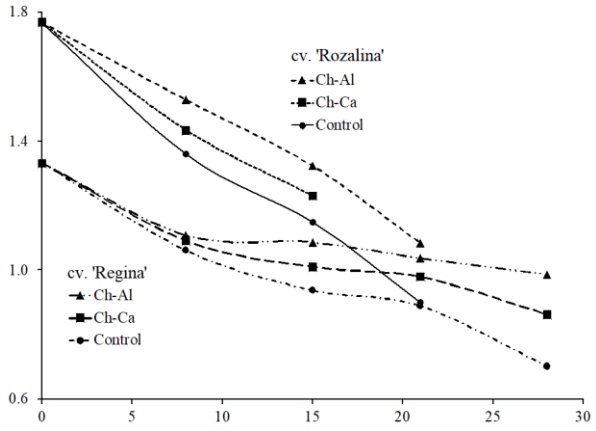

Figure 2 Crunchiness

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

Texture changes: The fresh sweet cherry fruits have high crunchiness (hard peel and flesh):

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

where F_f = yield force, F_r = rupture force, ℓ_f = yield deformation, ℓ_r = rupture deformation.

The crunchiness of the white cv. was higher during the storage time than the black cv. (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).

Soluble solid content: The TSS values of the coated and uncoated fruits increased over the 22 or 29 days of cold storage period (Table 1). The increasing could be attributed to the breakdown of

starch to sugar, to the decrease in respiration rate and conversion of sugars in CO_2 and H_2O (Ghasemnezhad et al. 2011), to the hydrolysis of cell wall polysaccharides (Comabella & Lara 2013), and to the increase of dry matter due to water loss (Petriccione et al. 2015). The changes is smaller in the coated samples, because the coatings are modified the internal atmosphere, reduce the respiration activity and the water-loss (Dong et al. 2004). According to our results, the Ch-Ca coating is slightly better in the preservation of the soluble solid content than the Ch-Al treatment.

Active acidity (pH): The active acidity was decreased (increased pH) significantly in the control samples during the shelf life. The increasing was reduced by chitosan based coatings (Table 1). The higher acidity loss in uncoated fruits could be the result of the use of organic acids as substrates for the respiratory metabolism (Díaz-Mula et al. 2009; Diaz-Mula et al. 2012). The acidity loss was slightly 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, peach, guava, and litchi – Hernandez-Munoz et al. 2008; Li & Yu 2001; Hong et al. 2012; Dong et al. 2004).

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 black cherry, but the decreasing is smaller for the white (Table 1). The shown cultivar dependence is known from other studies as well (Pasquariello et al. 2015). The results obtained show how to mitigate the coatings the decreasing of the antioxidant activity. The reducing effect of the Ch-Al bilayer coatings is higher than the Ch-Ca.

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

Conclusions: Based on the reported results the chitosan based coatings preserve the sweet cherry during the shelf-life period. Furthermore, both coating combinations delayed the quality deterioration, in cultivar dependent manner. The white sweet cherry cultivar is more sensitive for the manipulation and has shorter storage time. Based on the quality and safety parameters the shelf life period for the cultivar ‘Rozalina’ was 21 days and for the black sweet cherry, cultivar ‘Regina’

was 28 days. In some cases, the effect Ch-Ca coating is stronger, but the Ch-Al treated fruits showed better texture parameters.

Table 1. Result of physico-chemical and microbiological properties

Cult.	Treat	D	Brix	Antioxidants	pH	TNM	TYM
'Rozalina'	Cont-Al-Ca	0	15.6±0.10	2207.10±0.42	3.68±0.03	4.67±0.35	3.22±0.15
	Cont.	8	16.0±0.05	2170.88±0.33	3.92±0.03	5.22±0.48	3.50±0.18
	Cont.	15	17.5±0.05	2121.76±0.44	4.02±0.04	5.47±0.46	4.16±0.23
	Cont.	22	17.7±0.05	2040.88±0.55	4.08±0.02	5.99±0.61	4.50±0.24
	Ch-Al	8	15.5±0.10	2200.59±0.40	3.99±0.02	4.98±0.26	3.38±0.23
	Ch-Al	15	16.5±0.05	2181.18±0.30	3.96±0.01	5.15±0.26	3.50±0.26
	Ch-Al	22	16.8±0.10	2101.31±0.48	3.99±0.02	5.29±0.31	4.01±0.19
	Ch-Ca	8	16.3±0.10	2194.65±0.28	3.90±0.02	4.99±0.27	3.43±0.21
	Ch-Ca	15	16.5±0.10	2146.14±0.19	3.93±0.02	5.37±0.35	3.98±0.27
'Regina'	Cont-Al-Ca	0	17.2±0.05	2930.46±0.40	3.61±0.02	4.83±0.22	3.17±0.17
	Cont.	8	18.8±0.05	2808.49±1.19	3.65±0.04	4.93±0.2	3.53±0.17
	Cont.	15	18.5±0.10	2718.82±0.55	3.74±0.03	4.99±0.08	3.84±0.18
	Cont.	22	19.3±0.10	2637.73±1.50	3.93±0.02	5.29±0.12	4.32±0.16
	Cont.	28	21.2±0.05	2488.46±0.42	3.99±0.02	5.51±0.34	4.55±0.21
	Ch-Al	8	17.5±0.10	2866.06±0.21	3.63±0.02	4.87±0.17	3.36±0.2
	Ch-Al	15	17.3±0.05	2802.88±0.25	3.73±0.02	4.92±0.31	3.76±0.25
	Ch-Al	22	18.6±0.05	2776.46±0.68	3.77±0.02	4.99±0.13	4.01±0.37
	Ch-Al	28	19.0±0.10	2693.36±0.60	3.82±0.02	5.00±0.24	4.33±0.38
	Ch-Ca	8	16.8±0.05	2828.24±0.64	3.67±0.02	4.88±0.18	3.46±0.20
	Ch-Ca	15	16.8±0.05	2749.55±0.42	3.73±0.02	5.13±0.15	3.80±0.19
	Ch-Ca	22	17.5±0.10	2648.28±0.60	3.79±0.01	5.27±0.14	4.20±0.21
	Ch-Ca	28	18.4±0.10	2557.84±0.95	3.97±0.02	5.33±0.10	4.51±0.23

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