Effect of chitosan edible coating on sweet cherry post-harvest storage pathogens

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Abstract The chitosan extends the shelf life of treated fruit due to its ability to form a semipermeable coating by minimizing the rate of respiration and reducing water loss. As a nontoxic biodegradable material, as well as an elicitor, chitosan has the potential to become a new class of fruit preservatives. Sweet cherry cultivars were treated with Chitosan-Ca-lactate and Chitosan-alginate solutions. The samples from 'Rozalina' – new Fruit Growing Institute cultivars, El.17-37 'Tzvetina' and El.17-90 'Asparuh' – new selected hybrids and candidate-cultivars of Fruit Growing Institute-Plovdiv as well as 'Bing' and 'Regina' – standard cultivars and controls for comparison were refrigerated at 4°C for 28 days, up to the end of their shelf-life time. During the trial, the following diseases were detected *Monilinia* sp., *Botrytis cinerea*, *Penicillium* sp., *Alternaria* sp., *Rhizopus* sp. and mixed infections. On the 21st day, the Chitosan-alginate gave better results than Chitosan-Ca-lactate in all tested cultivars, and the best-preserved cultivar was 'Regina'.

Key words: Chitosan, *Prunus avium*, Diseases

Introduction

Sweet cherries are a fruit with a high transpiration rate and a susceptibility to fungal rots and physiological disorders (Alique et al. 2005). Harvested sweet cherry fruits are highly perishable and often do not reach consumers at an optimal quality. Cold storage is the main postharvest treatment to reduce the rate of many metabolic processes in perishable fruits, to maintain quality, and to extend the storability of cherries considered to be non-chilling sensitive. To extend the shelf life of postharvest fruit, recently, several technologies, including modified atmosphere packaging, irradiation, and coating in combination with cold storage, have been applied (Xanthopoulos et al. 2012; Castagna et al. 2013).Postharvest decay may result in serious economic losses to sweet cherries, a commodity of economic importance in many production areas worldwide. Fruit can be damaged by *Monilinia laxa* (brown rot), by *Botrytis cinerea* (grey mould) and, with a lower incidence, by *Alternaria alternata* (Alternaria rot), *Penicillium expansum* (blue mould), *Rhizopus stolonifer* (Rhizopus rot), and *Cladosporium* spp.(green rot) (Ippolito et al., 1997). The use of synthetic fungicides to control postharvest diseases of sweet cherries is not allowed by European legislation, and there is a need for alternative approaches.

Alternative methods for controlling storage rots include biocontrol agents (Ippolito et al., 1997b; Droby et al., 1999), natural substances (Wilson et al., 1997; Romanazzi et al., 1999). At present, preharvest treatments with synthetic fungicides are the main means for postharvest disease control in stone fruit in general. However, alternatives to the use of synthetic fungicides are needed for the sweet cherry market, where no fungicides are registered for postharvest applications and none are allowed in organic agriculture. Moreover, they have the potential to reduce the impact of agriculture on the environment and on human health (Elmer and Reglinski, 2006; Mari et al., 2010).

Chitosan is a natural polysaccharide biopolymer derived from chitin, the principal structural component of the crustacean exoskeleton. Depending on the degree of deacetylation, chitosan can have an extensive collection of C2 amino groups, having pKa values of ∼6.5, which can become protonated in weakly acidic conditions. It is this polycationic character that confers chitosan’s antimicrobial properties, which favours interaction with negatively charged microbial cell walls and cytoplasmic membranes. These interactions result in decreased osmotic stability, membrane disruption, and eventual leakage of intracellular elements (Ma et al.,2008; Banerje et al.,2010) In addition, chitosan may enter the nuclei of bacteria and fungi and inhibit mRNA and protein synthesis by binding to microbial DNA (Qi et al., 2005; Ma et al., 2008; Blecher et al., 2011). When nanoscaled, chitosan has a higher surface-to-volume ratio, translating into higher surface charge density, increased affinity to bacteria and fungi, and greater antimicrobial activity (Qi et al., 2005).

Materials and Methods

The effect of chitosan coatings on sweet cherry after harvest was observed during a storage period for disease control. Three sweet cherry cultivars were selected for this study Bing, Regina and Rosalina and two cherry elites Elit 17-37 'Tsvetina' and Elit 17-90 'Asparuh'. The fruits were collected in 2019 from trees grown under standard commercial practices in Fruit Growing Institute Plovdiv. Fruits were randomly harvested at the commercial ripening stage and were transported to the laboratory and screened for appearance and absence of physical defects or decay. Subsequently, the fruits were randomly distributed in three variants prior to treatment.

In this study were applicated with two edible coating Chitosan-Ca-lactate (multicomponent) and Chitosan-alginate (bi-layer). 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 were bought from Sigma Aldrich, Bulgaria.

During the experiment in the control variant, a fruit was washed with water. The variant with Chitosan-Ca-lactate (multicomponent) fruits was immersed in 1% solution after this a fruit was dried for 10 minutes in normal conditions. In a variant with Chitosan-alginate (bi-layer) washed cherry fruits was immersed in 1% solution low molecular weight chitosan solution for 10 minutes after this a fruit was dried for 10 min. Next step a fruit was immersed again in a 1% sodium alginate solution for 10 minutes and dried again for 10 min.

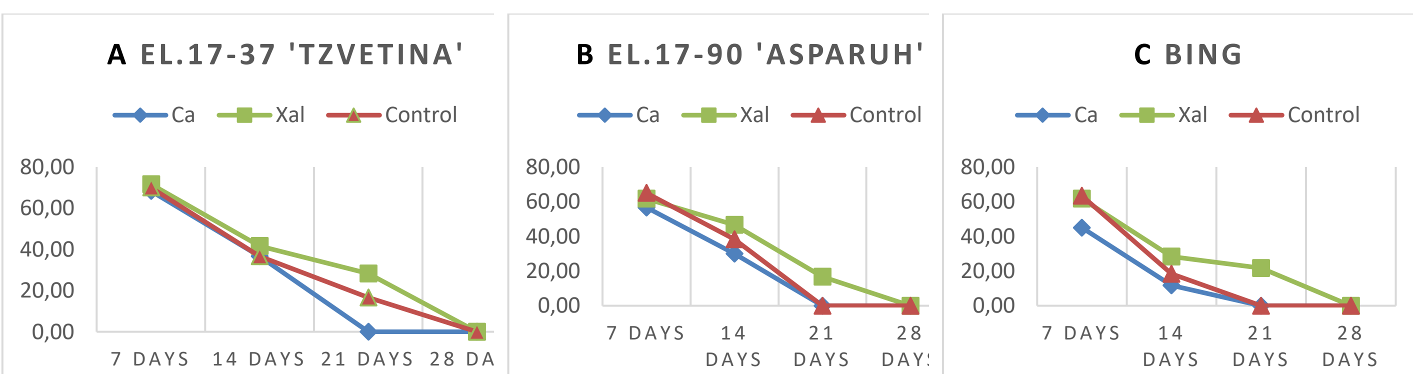
For each variants and control variant was used in 60 cherry fruits. During the 21 days storage period, 30 probes from each treatment were inspected 4 times based on a visual appearance and attack of rot diseases. To determine the disease attack was used percentage loss of infected cherry fruit: X = a.100 / A (X – loss in percentage (%), a – Infected fruits , A – The total number of plants/ fruits

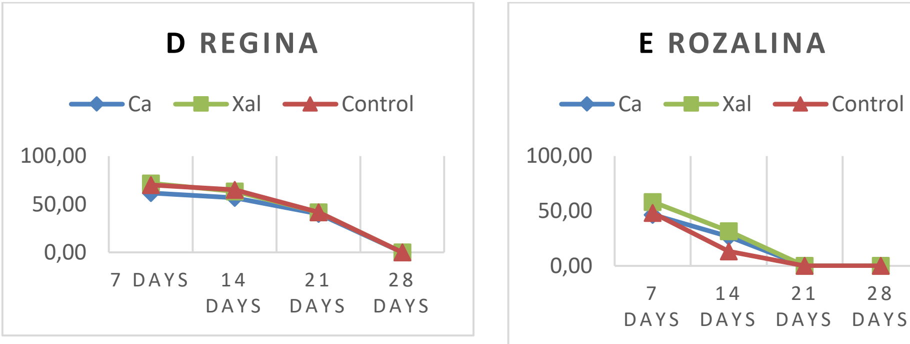
Data obtained were analyzed statistically using SPSS 19.0 program. Data were subjected to Duncan's analysis of variance (ANOVA) and means were separated by Duncan's multiple range tests at $p \leq 0.05$.

Results

During the storage period on the 7th days after treatment with chitosan-alginate at candidate-cultivar 'Tsvetina' and Regina cultivar we have reported the highest percentage of healthy fruits 71.67%. Among studied cultivars and elites used, the percentages of healthy fruits in the control variant are the highest Elite 17-90 'Asparuh' and Bing.

Figure 1 A to E – Infection progress in different cherry cultivars and hybrids (% of healthy fruits).



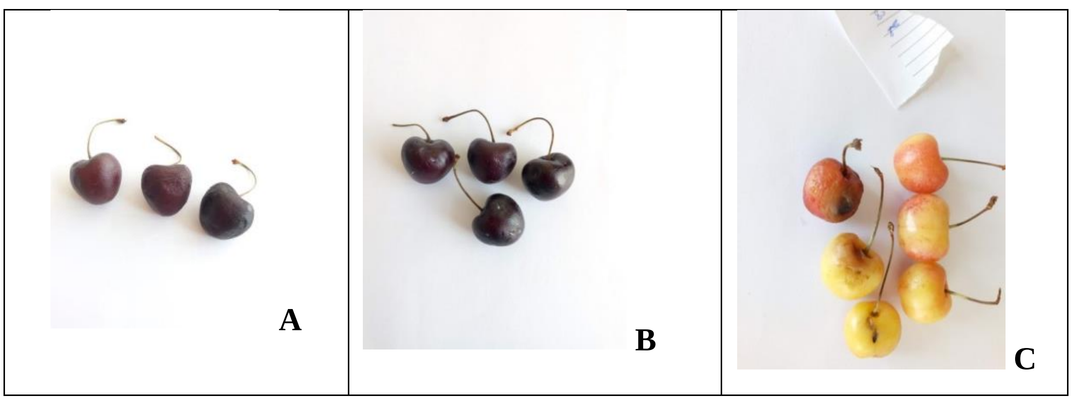


On the 14th day in all cultivars, cherry fruits treated with chitosan-alginate there is a tendency for them to have the highest values for healthy fruits, except for the Regina which cultivar, the control variant with higher results.

On 21th days after treatment with Chitosan-Ca- L in all variants with treated fruits are not successfully preserved, except for the ‘Regina’ cultivar with 40% healthy fruits. The variants treated with Chitosan-Alginate with the highest percentage healthy fruits are ‘Regina’ (41.67%) and candidate-cultivar 'Tsvetina' (28.33%). In the control variant also a high percentage is reported in ‘Regina’ cultivar.

On the 28th days none of the variants included healthy, uninfected cherry fruits.

Picture 1. A to C General look of fruits of fruits on the 21th day of storage.



Cultivars	***Monilinia sp.***			***Botrytis cinerea***			***Penicillium expansum***		
	ChCaL	**ChAl**	**Control**	**ChCaL**	**ChAl**	**Control**	**ChCaL**	**ChAl**	**Control**
El.17-37 'Tzvetina'	8,88 ab	10,55 b	9,44 b	11,11 a	11,66 a	9,44 a	5,56 a	0 b	8,88 b
El.17-90 'Asparuh'	4,44 ab	0 c	15,55 a	11,66 a	17,22 a	10,01 a	8,89 a	6,67 ab	3,33 c
Bing	6,11 ab	0 c	1,11 c	10,01 a	12,77 a	12,78 a	6,11 a	9,44 a	10,55 b
Regina	1,66 b	1,66 c	5,55 bc	10,55 a	11,67 a	7,22 a	6,67 a	10,01 a	16,11 a

Rozalina	13,33 a	18,33 a	2,22 c	13,88 a	0 b	8,89 a	0 b	0 b	9,44 b
	Alternaria alternata			***Rhizopus sp.***			***Mixed infection***		
	ChCaL	**ChAl**	**Control**	**ChCaL**	**ChAl**	**Control**	**ChCaL**	**ChAl**	**Control**
El.17-37 'Tzvetina'	0 b	0 a	1,66 ab	0 a	1,11 ab	1,11a	7,77 a	10 a	2,78 a
El.17-90 'Asparuh'	0,56 b	0 a	2,78 ab	0 a	0 b	1,66 a	7,22 a	9,44 a	0 b
Bing	0 b	0,56 a	0 b	0 a	0,56 ab	2,77 a	11,11 a	10 a	6,11 ab
Regina	2,77 a	0,56 a	0 b	0,56a	1,11 ab	1,66 a	10 a	8,33 a	2,77 ab
Rozalina	0 b	1,67 a	3,89 a	1,67 a	4,44 a	1,66 a	6,11 a	8,89 a	7,22 b

Table 1. Infected fruits (%) in different cherry cultivars and hybrids

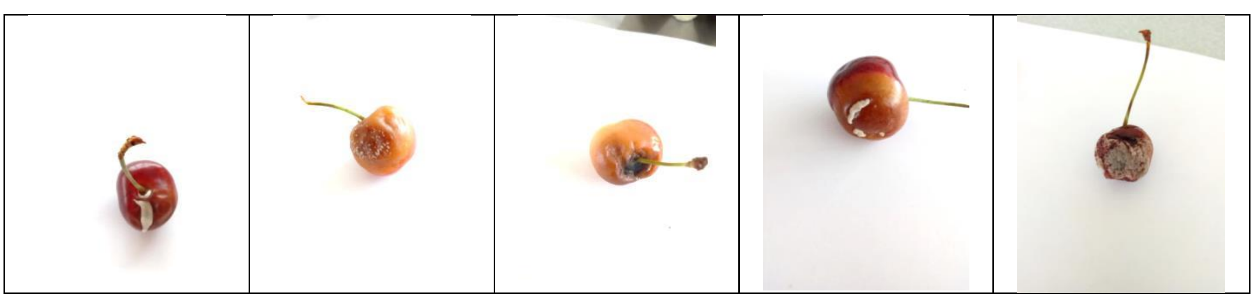
During the storage period of the cherry fruits, depending on the combination of cultivars and coating used, different diseases manifested symptoms.

The infected fruit by *Monilinia sp.* (brown rot) in variant with coating ChCaL, a low percentage of attack is reported in 'Regina' cultivar – 1.66%. In variant with Chitosan-Alginate coating, significant differences are reported between 'Tzvetina', 'Regina' and 'Rozalina' cultivars. In the non-treated El.17-90 'Asparuh' variant, infected fruits are 15.55% and statistically different from all other variants.

Infection caused by *Botrytis cinerea* (grey mould) almost in all variant of Chitosan coating is not significant, the only exception is 'Rozalina' cultivar coated with Chitosan-Alginate.

Similar results are reported in *Penicillium expansum* (blue mould) in variant with Chitosan-Ca, exception is cv.'Rozalina' – 0% infected fruits. In Chitosan-Alginate coating, result show statistically significant differences between El.17-90 'Asparuh', 'Bing', 'Regina' and El.17-37 'Tzvetina', 'Rozalina cultivars'. In control variant, cultivars were sorted in three groups: high infection - Regina cultivar; meduim infection - El.17-37 'Tzvetina' Bing and Rozalina; low infection - El.17-90 'Asparuh.

Picture 2. D to H Infected fruits, during the storage period



D	E	F	G	H

Alternaria rot caused by fungus *Alternaria alternate* in all variants with Chitosan-Alginate have no signification differences between cultivars. In the variants with Chitosan-Ca there are signification differences only in ‘Regina’ cultivar.

The result for *Rhizopus stolonifer* (Rhizopus rot) showed that there are no significant differences between cultivars coated with Chitosan-Ca- Lactad and the non-treated control, but in coated with Chitosan-Alginate have significant differences between ‘Rozalina’ and 'Asparuh'.

In mixed infections on fruits, similar results were obtained. Statistically proven differences were recorded in the non-treated control variant of El.17-90 'Asparuh’, ‘Rozalina’ and El.17-37 'Tzvetina'.

Table 2. Effect of coating of Chitosan about limitation of pathogens (%) in storage period

Diseases	**Variants**	**Cultivars**				
		El.17-37 'Tzvetina'	**El.17-90 'Asparuh'**	**Bing**	**Regina**	**Rozalina**
Monilinia sp.	**ChCaL**	8,88 a	4,44 b	6,11 a	1,67 a	13,33 a
	ChAl	10,55 a	0 c	0 b	1,67 a	18,33 a
	Control	9,44 a	15,55 a	1,11 b	5,56 a	2,22 b
Botrytis cinerea	**ChCaL**	11,11 a	11,66 a	10 a	10,55 a	13,89 a
	ChAl	11,66 a	17,22 a	12,78 a	11,67 a	8,89 b
	Control	9,44 a	10 a	12,78 a	7,22 a	0 c
Penicillium expansum	**ChCaL**	5,55 a	8,89 a	6,11 a	6,67 b	0 b
	ChAl	0 b	6,67 a	9,44 a	10 ab	0 b
	Control	8,88 a	3,33 a	10,55 a	16,11 a	9,44 a
Alternaria alternata	**ChCaL**	0 a	0,56 a	0 a	2,77 a	0 a
	ChAl	0 a	0 a	0,56 a	0,56 a	1,67 a
	Control	1,66 a	2,78 a	0 a	0,00a	3,89 a
Rhizopus sp.	**ChCaL**	0 a	0,56a	0 a	0,56 a	0 a
	ChAl	1,11 a	0 a	0,56 a	1,11 a	1,67 a
	Control	1,11 a	1,67 a	2,78 a	1,66 a	4,44 a
Mixed infection	**ChCaL**	7,77 a	7,22 a	11,11 a	10 a	6,11 a

	ChAl	10 a	9,44 a	10 a	8,33 a	8,89 a
	Control	2,77 b	0 b	6,11 a	2,78 a	7,22 a

Statistical analysis showed that almost in all the Chitosan coated non-statistically significant differences between a coated and control variant.

Conclusions

- During storage period, the coatings Chitosan -Alginate have preserved the fruits for a longest period
- The phytopathogenes attack of the cherry fruits depends on the combination of cultivars and coating used.
- The coatings of Chitosan inhibited the diseases caused by *Alternaria alternata* and *Rhizopus sp*.
- Application of 1% solution of chitosan coatings did not effectively limit the post-harvest diseases

This study demonstrates the potential of chitosan coatings as an antifungal preparation for cherry fruits that are quite susceptible to decay caused by fungal diseases. However, further studies are required to determine the optimal concentration of Chitosan for decay control of fungal diseases in the storage period.

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