



Postharvest Biopreservation of Fresh Blueberries by Propolis-Containing Edible Coatings Under Refrigerated Conditions

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Abstract

Propolis is a complex honey bee product known for its antioxidant potential and antimicrobial activity, widely used as a food biopreservative and food additive. The object of the current research was to investigate the effects of carboxymethyl cellulose (CMC) edible coatings applied alone and in combination with a propolis extract on the quality parameters and storage life of fresh blueberries during refrigerated storage for 20 days. For this purpose, three experimental groups were prepared: blueberries without coating (control group), blueberries with 1% CMC coatings and blueberries with 1% CMC coatings+1% propolis extract (CMC+P). During the storage, the physicochemical and microbiological parameters of the experimental groups were evaluated. The use of CMC and CMC+P coatings reduced the weight loss by 1.13% and 1.67% in comparison with the control group on the 20-th day of storage. A significant decrease in decay percentage was found, which was in the great extent in the CMC+P coated fruit compared to the CMC coatings and the control fruit. The CMC and CMC+P edible coatings did not affect the TSS levels, the decreasing TA and increasing pH values. The application of CMC and CMC+P coatings did not cause a protective effect on the lowering values of total phenolic and anthocyanin contents in both treatments, but exhibited a positive influence on the antioxidant activity in



Article History

Received: 09 November 2021

Accepted: 24 January 2022

Keywords

Blueberry; Biopreservation; Edible Coatings; Propolis.

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Doi: <http://dx.doi.org/10.12944/CRNFSJ.10.1.08>

the coated blueberries. During the entire storage period, propolis containing edible coatings (CMC+P) reduced the bacterial, yeasts and fungal counts, visibly expressed by a reduction in decay incidence in comparison with the uncoated and CMC-treated fruit. Therefore, the application of propolis in the composition of edible coatings can be considered as an effective approach for improving the postharvest quality and prolonging the storage life of fresh blueberries.

Introduction

Blueberry belongs to the genus *Vaccinium*, family *Ericaceae*- a widely spread group of perennial plants with blue or purple edible fruits.¹ Blueberries are commonly consumed fresh as a dessert fruit, in processed form (jam, puree, syrup, salads, yoghurts, smoothies and other beverages) or can be baked in a variety of pastries.² Hailed as a “superfood”, blueberries are an excellent source of dietary fibres, vitamin C, vitamin K, manganese, iron, and large amounts of biologically active compounds (flavonoids, phenolic acids, anthocyanins and tannins) that play role as antioxidants and possess nutritional properties and health benefits.³⁻⁵ They are known to have *in vitro* and *in vivo* anticancer, anti-inflammatory, anti-metastatic, cardio-protective, anti microbial, anti-diabetic, anti-aging, reno-protective, opthalgo-protective, hepatoprotective, gastro-protective, anti-osteoporotic, and prebiotic effects.^{6,7,2} Other studies revealed the neuroprotective potential of blueberry’s polyphenolics and anthocyanins, and their role in enhancing the memory and delaying the degenerative processes associated with the brain aging.⁸ The rich phytochemical composition and the great number of therapeutic effects, make blueberry fruit valuable ingredient for the production of functional food products.

The bioactive compounds in blueberry fruit are unstable and can undergo various structural, biochemical and nutritional modifications during the postharvest storage and distribution. Although these changes depend on certain factors (fruit cultivar, degree of maturity, harvest approach and conditions of storage), the storage life of blueberries is limited to 10-40 days, and this period can be additionally shortened by physical injuries, moisture loss and fungal decay, leading to deterioration of fruit quality and economic losses. To overcome these limitations and to reduce decay

incidence, different methods for fruit preservation have been developed such as refrigerated storage, modified atmosphere packaging and ozonation, UV irradiation, and application of some non-conventional methods such as edible coatings.^{9,10} Edible coatings are thin layers of biopolymers (polysaccharides, proteins, lipids or resins) that extend storage life of perishable fruit, preventing chemical, physical and microbiological changes.^{11,12} They prevent water loss, O₂, CO₂ and lipid transmission, and accordingly restrict the fruit dehydration, desiccation and deterioration of organoleptic properties.^{13,14} Edible coatings render additional beneficial effects on perishable fruits by reduction of respiration rate, improvement of texture, and preservation of natural color and volatiles, thus protecting the fruit quality and nutritional value.^{15,16} The most important factor for the efficiency of edible coatings is the nature of the coating material, which might be used singly or as a carrier of bioactive compounds such as antimicrobial substances, nutraceuticals, and antioxidants, in order to improve their fruit biopreservation properties.¹⁷

Propolis is one of the most valuable bee products, possessing a broad spectrum of biological activities. Propolis (bee glue) is a sticky substance that honey bees (*Apis mellifera* L.) produce by mixing exudates gathered from different plant sources (flowers, leaf buds, tree secretions) with bees wax and saliva. Worker bees use propolis mainly as a building material and to protect their hives from pathogens, invaders and environmental conditions. Currently, more than 300 chemical compounds have been identified as constituents of propolis.^{18,19} The main components are polyphenols (especially flavonoids), followed by aromatic acids and esters, aliphatic acids and esters, hydrocarbons, waxy acids, alcohols, aldehydes, ketones, amino acids, steroids, enzymes, volatile

compounds, micro- and macro nutrients, vitamins, essential oils, sugars, pollen and other organic components. Most of them are known to possess antimicrobial activity and antioxidant potential.²⁰⁻²² In this regard, the biological properties of propolis make this unique bee product proper for use in the food industry as a natural preservative, an additive, or an ingredient with good functionality. Numerous studies have revealed the potential of propolis as a biopreservative in the composition of edible coatings for blueberries,²³ strawberries,^{24,25} raspberries,²⁶ red chilli,²⁷ bell pepper,²⁸ table grapes,²⁹ tangerines,³⁰ figs,³¹ cherry tomatoes,³² bananas,³³ papaya,³⁴ tomatoes³⁵ and oranges.³⁶

Edible coatings containing propolis have not been extensively investigated in blueberry biopreservation, and the existing data in the scientific literature are still very limited. Therefore, the aim of the current study was to evaluate the effects of application of edible coatings made of carboxymethyl cellulose and propolis on the quality characteristics and improvement of the postharvest life of fresh blueberries during refrigerated storage.

Materials and Methods

Materials

Fruit

Fresh organic blueberries (*Vaccinium myrtillus*) were bought from the fruit market in town of Plovdiv, Bulgaria. The blueberry fruit was chosen based on shape, size, color, ripeness, and without physical damages. The blueberries were carefully put in paper bags, and then quickly transported to the laboratory using a fridge bag.

Propolis

Fresh propolis was purchased from a local producer of bee products from the town of Simitli, Blagoevgrad district, Bulgaria (41°53'N 23°7'E). The ethanolic propolis extract was prepared as described below.

Culture Media

Plate Count Agar (PCA)

PCA medium used for determination of the total plate count of mesophilic aerobic and facultative anaerobic microorganisms was prepared according to the instructions of the manufacturer (Scharlab S.L., Spain).

Chloramphenicol Glucose Agar (CGA)

CGA medium used for enumeration of yeasts and fungi was prepared according to the instructions of the manufacturer (Scharlab S.L., Spain).

Preparation of Propolis Extract

Propolis (4 g) was finely cut, placed in a plastic tube (Isolab, Germany), poured with 10 ml of 50% ethanol (Sigma-Aldrich, Merck, Germany), shaken using a vortex (Biosan, Latvia) for 20 s, and then left at 4°C for 48 h to extract, as the sample was periodically shaken. Next, the extract was filtered and then stored at 4°C until use.

Experimental Procedure

The 1% edible coating solution was prepared by the following prescription: 4 g of carboxymethyl cellulose powder (Dow Wolff Cellulosics GmbH, Germany) was dissolved in 400 ml of distilled water, homogenized using a magnetic stirrer (Isolab) at 800 rpm for 30-40 min, and then kept at 4°C for 24 h. Next, the edible coating solution was separated into two portions of 200 ml each. To obtain an edible coating solution with final propolis concentration of 1%, 5 ml of propolis extract was added to the second carboxymethyl cellulose (CMC) portion and stirred under the same conditions. Thus, two variants of edible coatings were prepared (CMC and CMC+P).³⁷

The blueberries were washed three times with tap water and air-dried at room temperature (23°C). Then, the blueberry fruit was randomly divided into three experimental groups of 330-350 g each: blueberries without coating (control), blueberries with 1% CMC coating (CMC) and blueberries with 1% CMC coating + 1% propolis (CMC+P). The blueberries were coated by immersion in the relevant coating-forming solutions for 3-5 min, drained of excess coating, and air-dried at room temperature (23°C). All experimental groups were stored at 4°C and 75% relative humidity (RH) for 20 days in plastic cups (Fig. 1) as previously described.^{37,38}

Quality Evaluations

All samples were analysed at 4-day intervals (days 0, 4, 8, 12, 16 and 20). During the entire monitoring period, the experimental groups were observed for decay changes and signs of

fungal growth. Samples for physicochemical and microbiological analyses in sterile containers were collected.^{37,38}

Weight Loss and Decay Percentage

Three separate experimental groups of 60-65 g blueberries each with identical treatments (control, CMC and CMC+P) were prepared and stored at 4°C and 75%RH. Each experimental group was weighed on days 0, 4, 8, 12, 16 and 20 of storage. Weight loss showed the percentage difference between the initial weight of each experimental group and the weight of the same group recorded on the relevant monitoring day. The decay percentage was calculated as the number of unacceptable fruit (with visible decay changes or fungal growth) in each experimental group divided by the initial number of all fruit multiplied by 100.^{37,38}

Physicochemical Analyses

Total Soluble Solids, Titratable Acidity, Ph And Maturity Index

The total soluble solids (TSS) content was determined by hand refractometer ATC (Aichose, China) and expressed as °Brix. The samples were preliminary homogenized by a blender MSM 14200 (Bosch, Germany), a few drops of blueberry juice were put on the prism glass, and the TSS value was immediately read. The titratable acidity (TA) was measured by titration of 2 ml of blueberry juice with 0.1 N NaOH (Sigma-Aldrich, Merck) using phenolphthalein as an indicator (Sigma-Aldrich, Merck) until the appearance of a pale pink color retained for over 1 min. The results were expressed as a citric acid percentage. The pH values were assessed by a pH-meter WTW pH 7110 (WTW, Germany) equipped with a glass electrode, which was immersed directly into the fruit juice at 23°C.^{37,38} Maturity index (MI) was determined as the TSS value was divided to TA.²³

Total Phenolic Content

The total phenolic content (TPC) was analyzed according to the standard method using Folin-Ciocalteu reagent.³⁷⁻³⁹ The results were presented as mg equivalent of gallic acid (GAE) per g of fresh weight (fw).

Total Anthocyanins Content

The total anthocyanins content (TAC) was determined using the standard pH differential method.^{37,38,40}

The results were presented as mg cyanidin-3-glycoside equivalents per g of fresh weight.

Antioxidant Activity

Dpph Radical-Scavenging Ability Assay

DPPH assay was performed following the standard method using 2, 2-diphenyl-1-picrylhydrazyl (DPPH reagent).^{38,41} The antioxidant activity was expressed as mM Trolox® equivalents (TE) per g of fresh weight.

Ferric Reducing Antioxidant Power (Frap) Assay

FRAP assay was assessed by the standard procedure.^{37,41} The antioxidant activity was presented as mM Trolox® equivalents (TE) per g of fresh weight.

Microbiological Analyses

Preparation of the Samples

Blueberries from each experimental group (10 g) were homogenized using a blender MSM14200 (Bosch). The samples (1 g) at an appropriate dilution in sterile 0.5% NaCl were pour-plated into PCA and CGA media. The Petri dishes (Gosselin™, France) were incubated at 30°/25°C for 24/48 h to measure the total plate count and yeasts and/or fungi.⁴²

Total Plate Count

The total plate count (mesophilic aerobic and facultative anaerobic microorganisms) was determined by colony-count technique on PCA at 30°C according to the Bulgarian State Standard BSS EN ISO 4833-1:2013.⁴³ The results were expressed as colony-forming units (cfu)/g.

Total Number of Yeasts and/or Fungi

The enumeration of yeasts and/or fungi was determined by colony-count technique on CGA at 25°C according to the Bulgarian State Standard BSS ISO 21527-1:2011.⁴⁴ The results were expressed as cfu/g.

Statistical Analysis

The results were performed in triplicates and presented as means ± standard deviation (SD). One-way analysis of variance (ANOVA) using Stat graphics Centurion statistical program (v. XVI, 2009) (StatPoint Technologies, Ins., Warrenton, VA, USA) was used. Mean differences were established by Fisher's test with a significance level $p \leq 0.05$.

Results and Discussion**Quality Evaluations and Physicochemical Analyses**

The results obtained for the decay percentage, weight loss percentage, pH values, TA, TSS, and

MI of all experimental groups (control blueberries, blueberries coated with 1% CMC, and blueberries coated with 1% CMC + 1% propolis) are summarized in Table 1.

Table 1. Decay, weight loss (WL), pH, titratable acidity (TA), total soluble solids (TSS), and maturity index (MI) of the experimental groups (control, blueberries coated with 1% CMC, and blueberries coated with 1% CMC + 1% propolis extract) during storage at 4°C for 20 days

Day	Sample	Decay, %	WL, %	pH	TA,% citric acid	TSS,°Brix	Maturity index
0	Control	-	-	2.91±0.01 ^{j,C}	0.99±0.00 ^{a,A}	10.00±0.00 ^{c,A}	10.10
	CMC	-	-	2.95±0.01 ^{i,B}	0.98±0.00 ^{b,B}	10.00±0.00 ^{c,A}	10.20
	CMC+P	-	-	2.98±0.01 ^{i,A}	0.97±0.00 ^{c,C}	10.00±0.00 ^{c,A}	10.31
4	Control	-	3.23±0.00 ^{l,A}	3.11±0.01 ^{h,B}	0.70±0.00 ^{d,A}	10.00±0.00 ^{c,A}	14.29
	CMC	-	3.22±0.00 ^{l,A}	3.17±0.01 ^{g,A}	0.65±0.00 ^{f,C}	10.00±0.00 ^{c,A}	15.38
	CMC+P	-	3.21±0.00 ^{l,A}	3.16±0.01 ^{g,A}	0.66±0.00 ^{e,B}	10.00±0.00 ^{c,A}	15.15
8	Control	-	5.72±0.00 ^{l,A}	3.28±0.01 ^{d,e,B}	0.62±0.00 ^{h,A}	10.00±0.00 ^{c,A}	16.13
	CMC	-	5.49±0.00 ^{l,B}	3.38±0.01 ^{c,A}	0.59±0.00 ^{k,B}	10.00±0.00 ^{c,A}	16.95
	CMC+P	-	5.40±0.00 ^{k,C}	3.27±0.03 ^{ef,B}	0.62±0.00 ^{h,A}	10.00±0.00 ^{c,A}	16.13
12	Control	-	9.40±0.00 ^{f,A}	3.37±0.03 ^{c,A}	0.59±0.00 ^{k,C}	10.00±0.00 ^{c,A}	16.95
	CMC	-	8.80±0.01 ^{g,B}	3.26±0.01 ^{ef,B}	0.62±0.00 ^{h,A}	10.00±0.00 ^{c,A}	16.13
	CMC+P	-	8.60±0.00 ^{h,C}	3.28±0.01 ^{e,B}	0.61±0.00 ^{j,B}	10.00±0.00 ^{b,A}	16.39
16	Control	-	13.88±0.01 ^{b,A}	3.31±0.03 ^{d,A}	0.60±0.00 ^{j,C}	11.50±0.00 ^{b,A}	19.17
	CMC	-	12.77±0.03 ^{d,B}	3.27±0.01 ^{ef,AB}	0.62±0.00 ^{h,B}	11.50±0.00 ^{b,A}	18.55
	CMC+P	-	12.24±0.03 ^{e,C}	3.24±0.00 ^{f,B}	0.63±0.00 ^{g,A}	11.50±0.00 ^{b,A}	18.25
20	Control	17.69 ^a	15.01±0.01 ^{a,A}	3.69±0.01 ^{a,A}	0.52±0.00 ^{n,C}	13.00±0.00 ^{a,A}	25.00
	CMC	10.46 ^b	13.88±0.01 ^{b,B}	3.63±0.03 ^{b,A}	0.54±0.00 ^{l,A}	13.00±0.00 ^{a,A}	24.07
	CMC+P	2.46 ^c	13.34±0.01 ^{c,C}	3.67±0.01 ^{a,A}	0.53±0.00 ^{m,B}	13.00±0.00 ^{a,A}	24.53

^{a-n}: Means in a column without a common letter differ significantly ($p \leq 0.05$).

^{A-C}: Means of the three samples on a given day without a common letter differ significantly ($p \leq 0.05$).



Fig. 1: Overall appearance of the experimental groups (control, CMC-coated blueberries and CMC+P- coated blueberries) at the beginning of the storage period (0 day)

Decay Percentage and Weight Loss Percentage

Besides the signs of desiccation, until the 16-th day of the refrigerated storage, morphological changes in any experimental group were not observed. After the 16-th day, the first visible changes of spoilage in all groups began to appear, and on the 20-th day of the observation period the decay percentage reached 17.69% in the control, 10.46% in CMC-coated blueberries, and 2.46%

in CMC+P-coated fruit (Fig. 2). The obtained results demonstrated that edible coatings containing propolis extract prevented most effectively the blueberry fruit from microbial activities and delayed the decay processes. The protective effect and the concomitant decrease in the decay percentage in the third experimental group (CMC+P) were related to the strong antimicrobial activity and antioxidant properties of propolis.

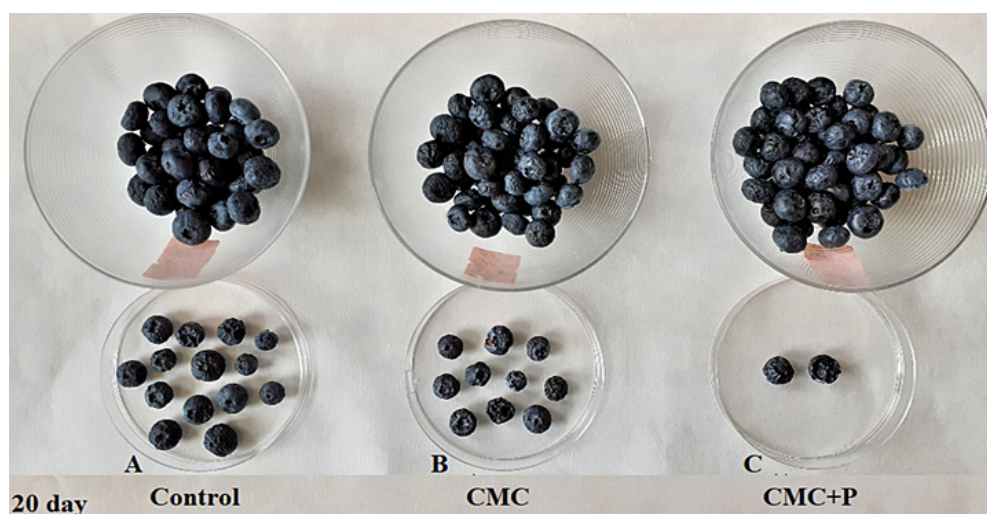


Fig. 2: Morphological changes (desiccation) on the 20-th day of the observation period. A, B, C (down) – blueberries with decay changes and visible fungal growth in the control, CMC- coated and CMC+P-coated experimental groups

All experimental groups demonstrated a progressive loss of weight during refrigerated storage (Table 1). On the 4-th day of the refrigerated storage, the weight loss (WL) was equal (3.2%) in all experimental groups. In the next days of the storage, the WL continued to increase gradually, which value was the highest in uncoated blueberries compared to the fruit with CMC and CMC+P coatings. At the end of monitoring period (day 20), the difference in the WL between the control blueberries and the treated fruit reached 1.13% (CMC) and 1.67% (CMC+P), respectively. Consequently, the application of 1% CMC edible coatings, especially in combination with 1% propolis extract (CMC+P) prevented desiccation and moisture loss in the treated blueberries until the end of the storage, thus helping to improve their storage life and overall appearance.

Changes in Physicochemical Parameters

Total soluble solids (TSS) are indicator for the fruit maturity rate. During the fruit ripening, metabolic processes are intensified, which leads to an increase in the sugar content. Titratable acidity (TA) and pH are the main parameters used for determining of the fruit quality during storage. As seen from the results in Table 1, a decrease in TA and a concomitant increase in pH values in all experimental groups were detected, normally associated with the processes of fruit ripening and postharvest changes. The obtained results demonstrated that CMC and CMC+P edible coatings did not consistently affect both parameters, which remained similar to the levels observed in the uncoated blueberries until the end of the refrigerated storage.

The total soluble solids expressed as °Brix, maintained constant levels in all treatments until the 12-th day of the storage under refrigerated conditions. On the 16-th day, an increase in TSS values in all experimental groups was detected, which raised gradually until the end of the storage period. This change was associated with the water transmission and subsequent fruit desiccation and weight loss. A proportional increase in maturity index in all treatments with prolongation of the storage period was also observed.

Similar findings were reported by other researchers, who examined the effects of pullulan coatings with addition of propolis extract on the extension of the storage life of fresh blueberries.²³ The authors stated that titrable acidity decreased and pH values increased in all treatments (control; pullulan coating; pullulan coating + 5% propolis extract, and pullulan coating + 10% propolis extract) with the prolongation of the storage time. During the storage, the TSS maintained higher levels in the control fruit, while the difference in WL percentage between four experimental groups varied within 0.4% (7-th day) and 2.18% (21-st day). The uncoated blueberries showed the highest values of WL. A decrease in titrable acidity and an increase in pH values were also found in blueberry fruit treated with chitosan-based edible coatings enriched with *Aloe vera* extract during cold storage at 5°C for 25 days.⁴⁵

Other works evaluated the effects of three types of coatings based on sodium alginate (Al), pectin (Pe) and sodium alginate + pectin (Al+Pe) on blueberry quality parameters and microbiological characteristics during cold storage for 14 days. The coated blueberries did not demonstrate significant differences in the WL, pH, and TSS levels. However, the application of edible coatings enhanced the fruit firmness and reduced the microbial growth as compared to the uncoated fruit.⁴⁶ The same authors observed similar effects in fresh blueberries coated with chitosan obtained from mushrooms in combination with procyanidins from grape seeds and stored under identical conditions.⁴⁷

Another effective approach in blueberry bio preservation is the application of edible coatings made of gum Arabic in combination with red and white roselle extracts during cold storage for 12 days.

The authors reported that the coatings inhibited the microbial growth, reduced effectively the WL and decay rates, delayed the anthocyanins and total phenolics degradation, and improved the fruit firmness. No statistically significant differences in the antioxidant activity between the coated and uncoated blueberries were detected. pH values and TSS levels in coated and control blueberries increased with the prolongation of the storage period.⁴⁸

The effectiveness of CMC-based edible coatings in combination with beeswax (30, 40 60%) on the quality of Mexican blueberry cultivar "Biloxi" when stored at 10°C for 21 days were also studied. The results demonstrated that after 7 and 14 days of storage, the blueberry fruit coated with 60% of bees wax had the lowest weight loss (2.28% and 4.03%, respectively) compared to the control fruit (3.8% and 7.42%, respectively). The authors observed no significant changes in TSS levels and TA values among all treatments over the storage period.⁴⁹

Changes in Total Phenolic Content (TPC)

Phenolic compounds are considered as non nutrient biologically active substances that determine the antioxidant potential of many plant species. Blueberries are one of the richest sources of compounds possessing antioxidant activity.⁵⁰ The results in Table 2 demonstrated that during the first four days of storage at 4°C and 75% RH, the TPC in both uncoated and coated blueberries maintained relatively high levels that were close to the initial polyphenolic concentration. However, after the 8-th day, the TPC gradually decreased in all experimental groups, reaching values of 0.93 mg GAE/g of fw (control), 1.01 mg GAE/g of fw (CMC) and 1.14 mg GAE/g of fw (CMC+P) on the 20-th day of the monitoring period. Through out the storage, the coated blueberries (CMC and CMC+P) maintained higher TPC values compared to the uncoated fruit. The edible coatings enriched with propolis extract (CMC+P) exhibited the highest protective effect on the TPC in coated blueberries during the entire storage period.

The decrease of TPC in blueberry fruit during storage at low temperatures was in agreement with the literature data. Chiabrando and Giacalone¹³ observed a reduction in the TPC in blueberries treated with three types of coatings - 2% chitosan,

1.5% sodium alginate, and 1.5% chitosan + 1% sodium alginate when stored at 0°C for 45 days. The declining trend in the TPC was confirmed by other authors, who examined four types of edible coatings based on 1% CMC, 0.3% xanthan gum,

0.75% guar gum and 10% gum Arabic on blueberry cultivar "Misty" when stored at 1°C and 85–90% RH. Although the TPC decreased, in both studies coated blueberries maintained higher levels of polyphenols in comparison with the control fruit.⁵¹

Table 2: Total phenolic content, total anthocyanins content and antioxidant activity of blueberries as influenced by edible coatings of 1% CMC and 1% CMC + 1% propolis extract (CMC+P) during storage at 4°C for 20 days

Day	Sample	Total phenols, mg GAE/gfw	Total anthocyanins, mg cyaniding-3- glyc/g fw	Antioxidant activity	
				DPPH, mM TE/gfw	FRAP, mM TE/gfw
0	Control	3.38±0.04 ^{a,A}	2.79±0.04 ^{a,A}	20.47±0.09 ^{ab,AB}	24.33±0.20 ^{ab,AB}
	CMC	3.29±0.01 ^{c,B}	2.74±0.07 ^{a,A}	20.25±0.28 ^{b,B}	24.02±0.04 ^{b,B}
	CMC+P	3.33±0.03 ^{b,AB}	2.76±0.18 ^{a,A}	20.94±0.06 ^{a,A}	24.69±0.11 ^{a,A}
4	Control	3.13±0.01 ^{d,B}	2.17±0.10 ^{b,A}	18.39±0.56 ^{d,B}	20.82±0.33 ^{gh,B}
	CMC	3.12±0.01 ^{d,B}	2.17±0.14 ^{b,A}	19.63±0.14 ^{c,A}	21.67±0.14 ^{e,B}
	CMC+P	3.30±0.01 ^{bc,A}	2.17±0.02 ^{b,A}	20.40±0.28 ^{b,A}	23.35±0.33 ^{c,A}
8	Control	2.08±0.01 ^{fg,B}	1.94±0.10 ^{bcd,A}	17.06±0.08 ^{e,B}	19.54±0.61 ^{l,B}
	CMC	2.11±0.01 ^{f,B}	1.92±0.13 ^{bcd,A}	16.44±0.37 ^{fg,B}	21.43±0.10 ^{ef,A}
	CMC+P	2.25±0.02 ^{e,A}	2.03±0.13 ^{bc,A}	20.09±0.29 ^{bc,A}	22.29±0.11 ^{d,A}
12	Control	1.93±0.02 ^{l,B}	1.94±0.12 ^{bcd,A}	16.46±0.28 ^{fg,A}	18.63±0.18 ^{i,C}
	CMC	1.97±0.02 ^{h,B}	1.93±0.15 ^{bcd,A}	16.19±0.28 ^{gh,A}	21.19±0.06 ^{fg,B}
	CMC+P	2.07±0.01 ^{g,A}	1.92±0.02 ^{bcd,A}	16.83±0.12 ^{ef,A}	21.78±0.04 ^{e,A}
16	Control	1.67±0.02 ^{k,B}	1.88±0.15 ^{bcd,e,A}	14.25±0.28 ^{j,B}	17.58±0.21 ^{k,C}
	CMC	1.68±0.01 ^{k,AB}	1.75±0.40 ^{cde,A}	15.64±0.06 ^{i,A}	19.56±0.06 ^{l,B}
	CMC+P	1.73±0.01 ^{j,A}	1.68±0.29 ^{cde,A}	15.89±0.02 ^{h,i,A}	20.37±0.21 ^{h,A}
20	Control	0.93±0.01 ^{n,C}	1.74±0.22 ^{cde,A}	10.91±0.29 ^{l,B}	17.20±0.11 ^{k,C}
	CMC	1.01±0.01 ^{m,B}	1.64±0.24 ^{de,A}	11.25±0.10 ^{l,B}	18.78±0.04 ^{l,B}
	CMC+P	1.14±0.01 ^{l,A}	1.54±0.13 ^{e,A}	12.55±0.28 ^{k,A}	19.51±0.29 ^{l,A}

^{a-n}: Means in a column without a common letter differ significantly ($p \leq 0.05$).

^{A-C}: Means of the three samples on a given day without a common letter differ significantly ($p \leq 0.05$).

Contrary to our results and other data existing in the literature, some researchers observed an increasing trend in polyphenolic concentrations in blueberries treated with pullulan coatings during storage at 4°C for 28 days and at 16°C for 14 days. According to the same authors, the TPC in the control and treated blueberries stored at 4°C increased by 9.66% and 11.70%, while the TPC in the control and treated fruit stored at 16°C increased by 21.62% and 20.75%, respectively. A gradual increase in the anthocyanin concentrations both in the control fruit and pullulan-coated blueberries irrespectively of the storage temperature was also detected.⁵²

Changes in Total Anthocyanins Content

Anthocyanins are known as pigments of the polyphenol class that confer red to blue color to many fruits and vegetables. The total anthocyanins gradually declined in all experimental groups and this trend was observed until the end of the storage period, regardless of the presence of edible coatings (Table 2). During the observation period, total anthocyanins kept similar concentrations in all groups, showing that the application of CMC and CMC+P coatings did not delay the anthocyanins degradation in blueberry fruit when stored at 4°C for 20 days. Lowered anthocyanins levels both in uncoated and treated blueberry fruit during

refrigerated storage was also reported by other authors, who studied four types of edible coatings based on 1% CMC and other hydrocolloids (xanthan gum, guar gum and gum Arabic) on the postharvest quality and storage life of "Misty" blueberries.⁵¹

Antioxidant Activity Changes

According to the results obtained by two methods, the antioxidant activity in all experimental groups gradually decreased throughout the storage period at 4°C for 20 days (Table 2). As seen from the results obtained by DPPH assay, the CMC and especially CMC+P treatments reduced the decrease in antioxidants compared to the control fruit. The results obtained by the FRAP method followed the same trend demonstrating that throughout the storage period the blueberries treated with CMC and CMC+P edible coatings maintained significantly higher antioxidant levels compared to the uncoated fruit. The edible coatings enriched with propolis extract (CMC+P) exhibited the highest protective effect on antioxidant activity, thus helping to prolong the storage life and enhancing the fruit quality and nutritional value. Despite the decrease of total polyphenolic and anthocyanins during the storage, the positive impact of CMC and CMC+P edible coatings on antioxidant activity could be explained with the protective effect on other bioactive compounds with antioxidant activity: hydrolysable tannins, vitamin C, vitamin E, carotenoids, enzymes and minerals, not determined in the present study.

Table 3: Correlation between the total phenolic content and antioxidant activity

Methods	Correlation, r^2
TPC / DPPH	0.9395
TPC / FRAP	0.8494
DPPH / FRAP	0.8663

The antioxidant activity correlates to the greatest extent with the total phenolic and anthocyanins contents, while vitamin C concentration has a limited contribution to the total antioxidant capacity (TAC).⁵³ The relationship between the antioxidant activity determined by the two methods (DPPH and FRAP) and the TPC values is presented in Table 3.

The obtained results revealed a high positive correlation r^2 between the TPC and antioxidant activity as follows: TPC and the DPPH assay - 0.9395; TPC and the FRAP assay - 0.8494; DPPH and FRAP assays - 0.8663. Consequently, the antioxidant activity is related to the TPC values of the examined blueberry fruit.

The correlation between the antioxidant activity determined by different methods, as well as the relationship between the TPC and TAC values were reported for 15 Brazilian cultivars of blueberry. The authors detected a high positive correlation between the results from three methods for antioxidant activity - ABTS/FRAP (0.94), DPPH/FRAP (0.86), and ABTS/DPPH (0.92), but a moderate correlation between the mean antioxidant activity (determined by the ABTS, DPPH, and FRAP) and the TPC and TAC values in the studied cultivars, varying as follows: ABTS/TAC and ABTS/TPC -0.64; DPPH/TAC -0.58; DPPH/TPC -0.77; FRAP/TAC - 0.69; FRAP/TPC - 0.63.⁵⁴ Another research revealed that TPC was highly correlated with TAC of four cultivars of blueberries grown in Northwest Croatia during 2006 and 2007. The authors stated that correlation coefficients varied as follows: DPPH/TPC: 0.84; ABTS/TPC: 0.78; FRAP/TPC: 0.85 (for blueberries harvested in 2006) and DPPH/TPC: 0.97; ABTS/TPC: 0.84; FRAP/TPC: 0.57 (for blueberries harvested in 2007), which demonstrated that studied parameters can vary greatly depending on the blueberry's cultivar and the plant growing season.⁵⁵

Changes in microbiological parameters

Total plate count

The number of mesophilic aerobic and facultative anaerobic microorganisms (the total plate count) in uncoated (control) and CMC-coated fruit increased during the storage period, reaching the highest values at the end of the monitoring period (20-th day (Table 4). The propolis extract in the edible coatings reduced the total plate count, and the blueberries treated with CMC with addition of propolis (CMC+P) retained significantly lower mesophilic aerobic and facultative anaerobic bacteria populations until the end of the storage, in comparison with the uncoated and CMC-coated fruits, which can be easily explained with the antimicrobial potential of the propolis extract.

Yeasts and Fungi

Throughout the storage period, an increasing trend in the number of yeasts and fungi both in the uncoated group and CMC-coated fruit was recorded. The addition of propolis extract in the composition of edible coatings (CMC+P) led to significant decrease in yeasts count, especially after the 8-th day of the storage compared to the uncoated fruit and single CMC-treatment, and this trend continued until the end of monitoring period. The addition of propolis as an edible coating ingredient (CMC+P) demonstrated good preservative effect also by inhibiting the

fungi compared to the control fruit and single CMC-treatment. The higher inhibitory effect of propolis extract on fungal growth was measured after the 8-th day of the refrigerated storage, visibly expressed by reduction in decay incidence in comparison with the uncoated and CMC-treated fruits (Table 4). Therefore, the application of propolis in the composition of edible coatings can be considered as a prospective mean for biopreservation and extending the storage life of fresh blueberry fruit.

Table 4: Microbiological parameters of blueberries coated with 1% CMC and 1% CMC + 1% propolis extract (CMC+P) during storage at 4°C for 20 days

Day	Sample	Parameters		
		TPC, cfu/g*	Yeasts, cfu/g	Fungi, cfu/g
0	Control	1.4x10 ⁴	2.0x10 ³	6.0x10 ²
	CMC	1.0x10 ⁴	1.5x10 ³	6.0x10 ²
	CMC+P	1.2x10 ⁴	1.0x10 ³	5.0x10 ²
4	Control	1.7x10 ⁴	6.0x10 ³	1.1x10 ³
	CMC	1.2x10 ⁴	3.5x10 ³	8.0x10 ²
	CMC+P	4.0x10 ⁴	2.4x10 ³	4.5x10 ²
8	Control	2.0x10 ⁴	1.1x10 ⁴	1.3x10 ³
	CMC	1.5x10 ⁴	1.0x10 ⁴	9.0x10 ²
	CMC+P	3.5x10 ³	4.0x10 ³	4.0x10 ²
12	Control	2.8x10 ⁴	3.0x10 ⁴	5.2x10 ³
	CMC	2.5x10 ⁴	1.0x10 ⁴	1.0x10 ³
	CMC+P	3.0x10 ³	3.0x10 ³	3.5x10 ²
16	Control	4.0x10 ⁴	6.0x10 ⁴	7.0x10 ³
	CMC	3.4x10 ⁴	5.0x10 ⁴	2.0x10 ³
	CMC+P	5.0x10 ³	2.0x10 ³	3.0x10 ²
20	Control	1.5x10 ⁵	7.0x10 ⁴	2.0x10 ⁴
	CMC	1.0x10 ⁵	6.5x10 ⁴	5.3x10 ³
	CMC+P	5.0x10 ⁴	1.5x10 ³	2.5x10 ²

* - TPC – Total plate count.

The postharvest storage life of blueberry fruit is limited by microbial, in particular fungal spoilage. The efficacy of propolis as an antimicrobial agent was reported in previous studies, which investigated the effects of pullulan-based edible coatings enriched with propolis extract on the improvement of the postharvest life and quality of fresh blueberries, and revealed that the addition of 5% and 10% ethanolic propolis extract in the composition

of edible coatings reduced bacterial and fungal counts by 3–4.5 log after 21 days of storage at 16°C and RH of 58–63%.²³ Some other authors evaluated the inhibitory effect of chitosan-based edible coatings on microbial growth in blueberry fruit by using nano-materials such as silicon and titanium dioxides⁵⁶ or in different combinations with other functional substances such as *Aloe vera*,⁴⁵ blueberry leaf extracts,⁴⁸ quinoa protein and

sunflower oil;⁵⁷ procyanidins extracted from grape seeds.⁴⁷ All studies showed reduction in microbial growth in treated fruits and revealed the potential of edible coatings to prolong the storage life and to preserve the nutritional value of fresh blueberries.

Conclusion

The present study demonstrated that the use of 1% CMC edible coatings singly and especially in combination with 1% propolis extract represents a safe, cheap and eco-friendly method to prolong storage life of fresh blueberries. The edible coatings with addition of propolis extract (CMC+P) effectively reduced the microbial growth, significantly delayed the decay process, limited the weight loss, thus preserving the health beneficial properties of treated blueberries, specifically the antioxidant activity when

stored at 4°C for 20 days. Our results suggested that incorporation of propolis in the edible coatings can find a successful practical application as a natural preservative for improving the quality and safety, and for extending the storage life of fresh blueberry fruit.

Acknowledgments

The authors declare no conflict of interests.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

References

- Mazza G. Compositional and Functional Properties of Saskatoon Berry and Blueberry. *Int J Fruit Sci.* 2005;5(3): 101–120.
- Patel S. Blueberry as functional food and dietary supplement: The natural way to ensure holistic health. *Mediterr J Nutr Metabol.* 2014;7(2): 133–143.
- Smith M. A. L., Marley K. A., Seigler D., Singletary K. W., Meline B. Bioactive Properties of Wild Blueberry Fruits. *J Food Sci.* 2000;65(2): 352–356.
- Starast M., Karp K., Vool E., Moor U., Tonutare T., Paal T. Chemical Composition and Quality of Cultivated and Natural Blueberry Fruit in Estonia. *Veg Crops Res Bulletin.* 2007;66:143-153.
- Kalt W., Cassidy A., Howard L. R., Krikorian R., Stull A. J., Tremblay F., Zamora-Ros R. Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. *Adv Nutr.* 2020;11:224–236.
- Schmidt B. M., Erdman J. W., Lila M. A. Effects of Food Processing on Blueberry Antiproliferation and Antioxidant Activity. *J Food Sci.* 2006;70(6): s389–s394.
- Neto C. C. Cranberry and blueberry: Evidence for protective effects against cancer and vascular diseases. *Mol Nutr Food Res.* 2007;51(6): 652–664.
- Andres-Lacueva C., Shukitt-Hale B., Galli R. L., Jauregui O., Lamuela-Raventos R. M., Joseph J. A. Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. *Nutr Neurosci.* 2005;8(2): 111–120.
- Duan J., Wu R., Strik B. C., Zhao Y. Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions. *Post harvest Biol Tec.* 2011;59(1): 71–79.
- Tahir H. E., Zhihua L., Mahunu G. K., Xiaobo Z., Arslan M., Xiaowei H., Yang Z., Mariod A. A. Effect of gum arabic edible coating incorporated with African baobab pulp extract on postharvest quality of cold stored blueberries. *Food Sci Biotechnol.* 2019; 1-10.
- Falguera V., Quintero J.P., Jimenez A., Munoz J. A., Ibarz A. Edible films and coatings: Structures, active functions and trends in their use. *Trends Food Sci. Tech.* 2011; 22(6): 292-303.
- Vieira J. M., Flores-López M. L., de Rodríguez D. J., Sousa M. C., Vicente A. A., Martins J. T. Effect of chitosan–Aloe vera coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit. *Postharvest Biol Tec.* 2016; 116: 88–97.
- Chiabrando V., Giacalone G. Anthocyanins, phenolics and antioxidant capacity after

- fresh storage of blueberry treated with edible coatings. *Int J Food Sci Nutr.* 2015;66(3): 248–253.
14. Cui H., Yuan L., Li W., Lin L. Edible film incorporated with chitosan and *Artemisia annua* oil nanoliposomes for inactivation of *Escherichia coli* O157:H7 on cherry tomato. *Int J Food Sci Tech.* 2016; 52(3): 687-698.
 15. Corbo M.R., Campaniello D., Speranza B., Bevilacqua A. and Sinigaglia M. Non-conventional tools to preserve and prolong the quality of minimally-processed fruits and vegetables. *Coatings.* 2015; 5(4): 931.
 16. Yang Z., Zou X., Li Z., Huang X., Zhai X., Zhang W., Shi J., Tahir H. E. Improved Postharvest Quality of Cold Stored Blueberry by Edible Coating Based on Composite Gum Arabic/Roselle Extract. *Food Bioprocess Tech.* 2019; 1-11.
 17. Pérez-Lavalle L., Carrasco E., Valero A. Strategies for Microbial Decontamination of Fresh Blueberries and Derived Products. *Foods.* 2020; 9(11): 1558.
 18. Sforzin J. M., Bankova V. Propolis: Is there a potential for the development of new drugs? *J Ethnopharmacol.* 2011;133(2): 253–260.
 19. Wagh V. D. Propolis: A Wonder Bees Product and Its Pharmacological Potentials. *Adv Phar Sc.* 2013; Article ID 308249: 1–11.
 20. Toreti V. C., Sato H. H., Pastore G. M., Park Y. K. Recent Progress of Propolis for Its Biological and Chemical Compositions and Its Botanical Origin. *Evid-Bas Complement Altern Med.* 2013; Article ID 697390: 1–13.
 21. Huang S., Zhan C.-P., Wang K., Li G., Hu F.-L. Recent Advances in the Chemical Composition of Propolis. *Molecules.* 2014; 19(12): 19610–19632.
 22. Pobiega K., Kraśniewska K., Gniewosz M. Application of propolis in antimicrobial and antioxidative protection of food quality – a review. *Trends Food Sci Tech.* 2019; 83: 53–62.
 23. Pobiega K., Igielska M., Włodarczyk P., Gniewosz M. The use of pullulan coatings with propolis extract to extend the shelf life of blueberry (*Vaccinium corymbosum*) fruit. *Int J Food Sci Tech.* 2020; 1-8.
 24. Thomas A. B., Nassur R. de C. M. R., Boas A. C. V., Lima L. C. de O. Cassava starch edible coating incorporated with propolis on bioactive compounds in strawberries. *Cienc Agrotec.* 2016;40(1): 87–96.
 25. Martínez-González M. del C., Bautista-Baños S., Correa-Pacheco Z. N., Corona-Rangel M. L., Ventura-Aguilar R. I., Del Río-García J. C., Ramos-García M. de L. Effect of Nanostructured Chitosan/Propolis Coatings on the Quality and Antioxidant Capacity of Strawberries During Storage. *Coatings.* 2020;10(2): 90.
 26. Moreno M. A., Vallejo A. M., Ballester A.-R., Zampini C., Isla M. I., López-Rubio A., Fabra M. J. Antifungal edible coatings containing Argentinian propolis extract and their application in raspberries. *Food Hydrocolloids.* 2020;107: 105973.
 27. Ali A., Chow W. L., Zahid N., Ong M. K. Efficacy of Propolis and Cinnamon Oil Coating in Controlling Post-Harvest Anthracnose and Quality of Chilli (*Capsicum annum* L.) during Cold Storage. *Food Bioprocess Tech.* 2013;7(9): 2742–2748.
 28. Ali A., Wei Y. Z., Mustafa M. A. Exploiting Propolis as an Antimicrobial Edible Coating to Control Post-harvest Anthracnose of Bell Pepper. *Packag Technol Sci.* 2014;28(2): 173–179.
 29. Pastor C., Sánchez-González L., Marcilla A., Chiralt A., Cháfer M., González-Martínez C. Quality and safety of table grapes coated with hydroxypropylmethyl-cellulose edible coatings containing propolis extract. *Postharvest Biol Tec.* 2011;60(1): 64–70.
 30. Putra R. E., Reizandy F., Faizal A., Kinasih I. Efficacy of Local Propolis as Edible Coating of Tangerine cultivar Garut (*Citrus reticulata* Blanco). *IOP C Ser Earth Env.* 2018;187: 012025.
 31. Aparicio-García, P. F. Ventura-Aguilar R. I., del Río-García J. C., Hernández-López M., Guillén-Sánchez D., Salazar-Piña D. A., de L. Ramos-García M., Bautista-Baños S. Edible Chitosan/Propolis Coatings and Their Effect on Ripening, Development of *Aspergillus flavus*, and Sensory Quality in Fig Fruit, during Controlled Storage. *Plants.* 2021;10(1): 112.
 32. Pobiega K., Przybył J. L., Żubernik J., Gniewosz M. Prolonging the Shelf Life of Cherry Tomatoes by Pullulan Coating

- with Ethanol Extract of Propolis During Refrigerated Storage. *Food Bioprocess Tech.* 2020; 13: 1447–1461.
33. Passos F. R., Mendes F. Q., da Cunha M. C., Pigozzi M. T., de Carvalho A. M. X. Propolis extract in postharvest conservation banana “prata”. *Rev Bras Frutic.* 2016;38(2): 1-11.
34. Barrera E., Gil J., Restrepo A., Mosquera K., Durango D. A coating of chitosan and propolis extract for the postharvest treatment of papaya (*Carica papaya* L. cv. Hawaiiana). *Rev Fac Nac Agr.* 2015;68(2): 7667–7678.
35. Putra R. E., Khairannisa S., Kinasih, I. Effect of Application Propolis as Biocoating on the Physical and Chemical Properties of Tomatoes Stored at Room Temperature. *IOP C Ser Earth Env.* 2017; 58: 012026.
36. Passos F. R., Mendes F. Q., da Cunha M. C., da Cunha M. C., de Carvalho A. M. X. Propolis extract coated in “Pera” orange fruits: An alternative to cold storage. *Afr J Agric Res.* 2016;11(23): 2043-2049.
37. Tumbarski Y., Nikolova R., Petkova N., Ivanov I., Lante A. Biopreservation of fresh strawberries by carboxymethyl cellulose edible coatings enriched with a bacteriocin of *Bacillus methylotrophicus* BM47. *Food Technol Biotechnol.* 2019; 57(2): 230-237.
38. Tumbarski Y., Petkova N., Todorova M., Ivanov I., Deseva I., Mihaylova D., Ibrahim S.A. Effects of pectin-based edible coatings containing a bacteriocin of *Bacillus methylotrophicus* BM47 on the quality and storage life of fresh blackberries. *Ital J Food Sci.* 2020; 32(2): 420-437.
39. Stintzing F.C., Herbach K.M., Mosshammer M.R., Carle R., Yi W., Sellappan S., Akoh C.C., Bunch R., Felker P. Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia* spp.) clones. *J Agric Food Chem.* 2005;53(2): 442-451.
40. Lee J., Durst R.W., Wrolstad R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J AOAC Int.* 2005; 88(5): 1269-1278.
41. Ivanov I.G., Vrancheva R.Z., Marchev A.S., Petkova N.T., Aneva I.Y., Denev P.P., Georgiev V.G., Pavlov A.I. Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species. *Int J Curr Microbiol App Sci.* 2014;3(2): 296-306.
42. Tumbarski Y., Pashova D., Petkova N., Ivanov I., Deseva I., Mihaylova D. Effects of a bacteriocin of *Bacillus methylotrophicus* strain BM47 and pasteurization on the storage life of fresh tomato juice. *J HygEng Design.* 2020;33: 65-75.
43. Bulgarian State Standard BSS EN ISO 4833-1:2013. “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”.
44. Bulgarian State Standard BSS ISO 21527-1:2011. “Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95”.
45. Vieira J. M., Flores-López María L., de Rodríguez Diana Jasso, Sousa Maria C., Vicente António A., Martins Joana T. Effect of chitosan–Aloe vera coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit. *Postharvest Biol Tec.* 2016;116: 88–97.
46. Mannozi C., Cecchini J. P., Tylewicz U., Siroli L., Patrignani F., Lanciotti R., Rocculi P., Dalla Rosa M., Romani S. Study on the efficacy of edible coatings on quality of blueberry fruits during shelf-life. *LWT - Food Sci Technol.* 2017;85: 440–444.
47. Mannozi C., Tylewicz U., Chinnici F., Siroli L., Rocculi P., Dalla Rosa M., Romani S. Effects of chitosan based coatings enriched with procyanidin by-product on quality of fresh blueberries during storage. *Food Chem.* 2018;251: 18–24.
48. Yang G., Yue J., Gong X., Qian B., Wang H., Deng Y., Zhao Y. Blueberry leaf extracts incorporated chitosan coatings for preserving postharvest quality of fresh blueberries. *Postharvest Biol Tec.* 2014;92: 46–53.
49. Rodríguez-Nieto J., Jarquín-Enríquez L., Vázquez-Celestino D., Rivera-Pastrana D., Mercado-Silva E., Dufoo-Hurtado D. Effect of the application of an edible coating on the quality of blueberry (*Vaccinium corymbosum*) during postharvest

- storage. *Acta Hort.* 2019;1265: 241–246.
50. Sellappan S., Akoh C. C., Krewer G. Phenolic Compounds and Antioxidant Capacity of Georgia-Grown Blueberries and Blackberries. *J Agr Food Chem.* 2002;50(8): 2432–2438.
51. Totad M. G., Sharma R. R., Sethi S., Verma M. K. Effect of edible coatings on “Misty” blueberry (*Vaccinium corymbosum*) fruits stored at low temperature. *Acta Physiol Plant.* 2019;41(183).
52. Kraśniewska K., Ścibisz I., Gniewosz M., Mitek M., Pobiega K., Cendrowski A. Effect of Pullulan Coating on Postharvest Quality and Shelf-Life of Highbush Blueberry (*Vaccinium corymbosum* L.). *Materials.* 2017;10(8): 965.
53. Kalt W., McDonald J. E., Donner H. Anthocyanins, Phenolics, and Antioxidant Capacity of Processed Lowbush Blueberry Products. *J Food Sci.* 2000;65(3): 390–393.
54. Rodrigues E., Poerner N., Rockenbach I. I., Gonzaga L. V., Mendes C. R., Fett R. Phenolic compounds and antioxidant activity of blueberry cultivars grown in Brazil. *Food Sci Technol* (Campinas). 2011;31(4): 911–917.
55. Dragovic-Uzelac V., Savic Z., Brala A., Levaj B., Kovacevic D. B., Bisco A. Evaluation of Phenolic Content and Antioxidant Capacity of Blueberry Cultivars (*Vaccinium corymbosum* L.) Grown in the Northwest Croatia. *Food Technol Biotech.* 2010; 48(2): 214–221.
56. Li Y., Rokayya S., Jia F., Nie X., Xu J., Elhakem A., Almatrafi M., Benajiba N., Helal M. Shelf-life, quality, safety evaluations of blueberry fruits coated with chitosan nano-material films. *Sci Rep.* 2021;11(55): 1-10.
57. Abugoch L., Tapia C., Plasencia D., Pastor A., Castro-Mandujano O., López L., Escalona V. H. Shelf-life of fresh blueberries coated with quinoa protein/chitosan/sunflower oil edible film. *JSci Food Agr.* 2015;96(2): 619–626.