PAPER

EFFECTS OF PECTIN-BASED EDIBLE COATINGS CONTAINING A BACTERIOCIN OF *BACILLUS METHYLOTROPHICUS* BM47 ON THE QUALITY AND STORAGE LIFE OF FRESH BLACKBERRIES

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ABSTRACT

The aim of the current research is to investigate the effects of edible coatings based on celery pectin singly and in combination with a bacteriocin of *Bacillus methylotrophicus* BM47 on the quality and storage life of fresh blackberries under refrigeration conditions. In this study three experimental groups were prepared: uncoated blackberries as a control, blackberries with 1% pectin coatings and blackberries with 1% pectin coatings+bacteriocin of *B. methylotrophicus* BM47. During the storage at 4°C and 75% RH for 16 days, the weight loss, decay percentage, total soluble solids (TSS), titratable acidity (TA), pH, organic acids, sugars, total phenolic content, total anthocyanins and antioxidant activity were analyzed.

The results showed that the application of pectin and pectin+bacteriocin coatings led to a reduction in weight loss with 6.3% and 6.7% compared to the control fruit on the 16-th day of storage. A decrease in decay percentage was also noticed, which was most pronounced in the pectin+bacteriocin coated fruit compared to the pectin coatings and control. The pectin and pectin+bacteriocin coatings reduced TSS levels with 0.4% and 0.6%, respectively compared to the control on the 16-th day of the storage, but did not affect TA and pH values. The pectin and pectin+bacteriocin coatings had no effect on decreasing total phenolic and anthocyanin contents or the concentration of sugars (glucose and fructose) in both treatments and the control fruit. The pectin and pectin+bacteriocin edible coatings exhibited a protective effect on the ascorbic acid content, maintaining concentrations of 57.5 mg/100 g of fw and 58.8 mg/100 g of fw (day 16), which were close to the initial values. The pectin and pectin+bacteriocin treatments had also a positive impact on antioxidant activity in the coated blackberries. Both edible coatings effectively inhibited its decrease with the prolongation of the storage time and kept antioxidant levels of 231.8 TE/100 g of fw and 232.4 TE/100 g of fw (day 16) that were close to the initial values.

Keywords: *Bacillus methylotrophicus*, bacteriocin, biopreservation, blackberry, edible coatings

1. INTRODUCTION

Blackberry (*Rubus fruticosus*) is a perennial plant in the family *Rosaceae* whose native populations grow primarily in the Mediterranean region of Europe. The cultivated blackberry is grown all over the world, and in recent years, worldwide production and consumption of blackberries continue to increase (STRIK *et al.*, 2007). The blackberry fruit is an excellent source of antioxidants, including various phenolic compounds (phenolic acids, tannins, stilbenes, flavonoids and anthocyanins) and ascorbic acid as well as large amounts of other compounds such as vitamins, minerals, fibers and volatiles (DE SOUZA *et al.*, 2014). The rich phytochemical composition and nutritional characteristics of blackberry fruit have been shown to exert a positive impact on human health. For example, the high levels of antioxidants in blackberries have been found to exhibit anti-inflammatory, anticarcinogenic, antimutagenic and antimicrobial properties (GYAWALI and IBRAHIM, 2014). The bioactive components in blackberries also reduce cholesterol levels, cause an analgesic effect and help to strengthen blood vessels (NILE and PARK, 2014). Some recent studies have shown that blackberry polyphenols exhibit a neuroprotective effect by delaying degenerative brain processes (TAVARES *et al.*, 2012).

On the other hand, blackberry is a highly perishable fruit with a very short market life. The storage time of blackberries after harvesting is limited due to their high susceptibility to physical injuries, desiccation and fungal spoilage caused by *Botrytis cinerea*, *Rhizopus* sp. and *Mucor* sp. which results in a rapid loss of commercially acceptable appearance. These post-harvest changes can be suppressed by storage at low temperatures or by creating modified atmospheres (lower oxygen and increased carbon dioxide levels) that delay tissue senescence. Advanced technologies such as ozone treatment and gamma radiation can also be used to decrease microbial decay and prolong the storage life of blackberries (MAFTOONAZAD *et al.*, 2007; OLIVEIRA *et al.*, 2013).

The conservation, distribution and marketing of blackberries can also be significantly improved through some non-conventional methods for extending the storage time such as the application of thin layers of biopolymers known as edible coatings, which protect the fruit from physical injuries, chemical and microbiological activities (FALGUERA *et al.*, 2011). Edible coatings and films act as barriers against moisture loss, oxygen, carbon dioxide and lipid transfer, thus preventing dehydration, desiccation and deterioration of the fruit quality (CUI *et al.*, 2016). Edible coatings provide additional benefits in perishable fruits by reducing respiration rate, improving textural quality, and helping to retain natural color and volatiles, thereby protecting the fruit's nutritional value (CORBO *et al.*, 2015).

The nature of the edible coating material is essential for the coating's effectiveness. Existing data in the literature show that hydrocolloids (polysaccharides and proteins) and lipids are among the most suitable structural matrices for edible coatings production. Most edible coatings include cellulose and its derivatives, starch, alginate, chitosan and pectin. Numerous studies have described and evaluated the protective effects of cellulose derivatives, starch, alginate and chitosan, but research on the application of pectin-based edible coatings is very limited (MAHAJAN *et al.*, 2018).

Pectins are biopolymers comprised of (1→4) α-D-galactopyranosyluronic acid units naturally esterified with methanol. Based on the content of methyl esters or the degree of esterification (DE) that has a decisive effect on solubility and gel formation properties, pectins are divided into two groups – high-methoxylated (DE>50%) and low-methoxylated (DE<50%) pectins (CORBO *et al.*, 2015). Pectins are also polysaccharides that are commonly used as gelling agents in the food industry. As such, pectins are of great interest as edible coating agents in fruit biopreservation due to their unique colloidal characteristics, strong gel formation properties and ability to provide an excellent oxygen barrier and aroma preservation. However, the primary disadvantage of all polysaccharide-based coatings is that they do not ensure a good moisture barrier due to their hydrophilic nature (OMS-OLIU *et al.*, 2008; VALDÉS *et al.*, 2015).

Berries contain high levels of sugars, other nutrients and water that make them an excellent substrate for microbial growth. In addition, the low pH of berry fruits is a prerequisite for fungal spoilage, which affects product quality, storage and distribution, resulting in significant economic losses (TOURNAS and KATSOUDAS, 2005). The resistance of some fungi to conventional fungicides and the proven negative effects of chemical treatments on human health have led to many limitations in the use of such chemicals. Consequently, research efforts have become more focused on the development of safer preservation methods that employ non-toxic biologically active compounds. Thus, the biological control of spoilage by antimicrobial substances such as bacteriocins synthesized by *Bacillus* sp. and lactic acid bacteria (LAB) are a promising alternative to the traditional fungicide application. Bacteriocins are safe; they do not alter the organoleptic characteristics of food and can be applied directly or indirectly by *in situ* production. Some LAB bacteriocins have been tested and have already shown promising potential against microbial spoilage of fruits and fruit products (BARBOSA *et al.*, 2017). Other bacteriocins with strong antifungal activity such as those produced by *Bacillus methylotrophicus* BM47 might also be prospective candidates for solving the problems associated with fungal decay (TUMBARSKI *et al.*, 2018).

The current study thus aimed to examine the application of edible coatings based on celery pectin alone and in combination with a bacteriocin synthesized by *Bacillus methylotrophicus* BM47 on the quality and extension of the storage life of fresh blackberry fruit.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Fruit

Fresh blackberries (*Rubus fruticosus*) were purchased from the local fruit market in Plovdiv, Bulgaria. The fruit was selected based on size, shape, color and absence of physical injuries. The blackberries were placed in brown paper bags and then immediately transferred into a fridge bag (7°C) to the laboratory to conduct the experiments.

2.1.2 Pectin

Low-methoxylated pectin was extracted from celery tubers (*Apium graveolens* var. *rapaceum* D.C.) purchased from the local fruit market in Plovdiv, Bulgaria. The tubers were washed with tap water, checked for impurities, and then peeled, coarsely chopped and dried at 40°C. Before extraction, the tubers were finely ground in a laboratory homogenizer and pre-washed with 70% ethanol acidified with 2% hydrochloric acid in order to obtain alcohol insoluble solids. Pectin was extracted by ultrasound-assisted extraction with 2% aqueous ammonium oxalate using the method of PETROVA *et al.* (2017). The degree of esterification (DE) and anhydrouronic acid content (AUAC) were determined by titration method (PETROVA *et al.*, 2017). The degree of acetylation (DA) was measured by the hydroxamic acid reaction method with β-D-glucose pentaacetate as a standard. All chemicals were purchased from Sigma-Aldrich, Merck (St. Louis, MO, USA). The molecular mass (Mm) was assessed by high-performance size-exclusion chromatography (HPSEC) analysis. Separation was conducted using an Elite LaChrom HPLC system (VWR™ Hitachi, Tokyo, Japan) coupled with a column Shodex OH-pack 806M (8 mm × 300 mm) and a refractive index detector Chromaster 5450 with an aqueous 0.1 M sodium nitrate solution at a flow rate of 0.8 mL/min (OGNYANOV *et al.*, 2018). The obtained celery pectin was characterized as low-esterified pectin with DE 46%, DA 2%, AUAC 70% and average Mm of 912694 g/mol.

2.1.3 Bacteriocin

A bacteriocin synthesized by the strain *Bacillus methylotrophicus* BM47 (previously isolated from a natural thermal spring in the Haskovo region of Bulgaria) was used in the experiment. The bacteriocin, purified by fast protein liquid chromatography (FPLC), contained an antimicrobial peptide with Mm of 19578 Da as characterized in an earlier research (TUMBARSKI *et al.*, 2018).

2.2. Methods

2.2.1 Experiment design

The edible coating solution (1%) was prepared by dissolving 4 g of celery pectin in 400 mL of distilled water at 45°C in a magnetic stirrer IKA® RCT classic (IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 800 rpm for 30 min. Next, 0.5% glycerol monostearate Cutina® GMS (Henkel, Düsseldorf, Germany) was added as a plasticizer, and the solution was stirred under identical conditions as outlined above for 15 min. The pectin solution was then divided into two equal parts of 200 mL each. After cooling to room temperature, 100 AU/mL (0.15 mg/mL) of the purified bacteriocin of *B. methylotrophicus* BM47 was added to the second part and stirred without heating for 15 min (TUMBARSKI *et al.*, 2019).

The blackberries were disinfected by being dipped in 1% sodium hypochlorite (Sigma-Aldrich, Merck, Germany) for 3 min, then washed three times with tap water and dried at 25°C for 2 h in a forced air MLW drying chamber (Labexchange, Burladingen, Germany). After drying, the blackberries were separated into three experimental groups of 30 berries each as follows: uncoated blackberries as a control (group 1), blackberries with pectin coatings (group 2) and blackberries with pectin coatings + bacteriocin (group 3). The blackberries from group 2 and group 3 were immersed in the relevant coating solutions for 2-3 min. The treated fruits were then dried at 25°C for 2 h in a drying chamber with forced air MLW (Labexchange), after which all groups were placed in plastic boxes and stored under refrigeration conditions (4°C and 75% RH) for 16 days (TUMBARSKI *et al.*, 2019).

2.2.2 Visual observation

All groups were examined at the beginning of the experiment (i.e. 0 day) and on the 4-th, 8-th, 12-th and 16-th day of storage. During the storage period, an observation of the morphological changes and fungal growth was made, and samples for analyses were taken.

2.2.2.1 Weight loss percentage

For determination of weight loss (WL) and decay percentage, three separate groups of 10 blackberries each with the same treatments - control, pectin and pectin + bacteriocin were prepared and stored under identical conditions. To measure WL, each group was weighed at the beginning of the experiment (i.e. 0 day) and on the 4-th, 8-th, 12-th and 16-th day of storage. WL was defined as the difference between the initial weight of each experimental group and the weight of the same group determined on the relevant monitoring day. The results were calculated as a percentage loss of the initial weight (TUMBARSKI *et al.*, 2019).

2.2.2.2 Decay percentage

The decay percentage was determined as follows: the number of berries with visible decay or morphological changes was expressed as a percentage of the initial number of all berries in the relevant experimental group (TUMBARSKI *et al.*, 2019).

2.2.3 Physico-chemical parameters

2.2.3.1 Total soluble solids, titratable acidity and pH

The total soluble solids (TSS) content was determined by a portable Abbe refractrometer (Officine Galileo, Campi Bisenzio, Italy). The samples were preliminary homogenized with a special device Polytron (Kinematica AG, Luzern, Switzerland), a few drops of blackberry juice were put on the prism glass, and the TSS value was immediately read and recorded. The titratable acidity (TA) was measured by titration of 2 mL of blackberry juice with 0.1 N NaOH (Sigma-Aldrich, Merck) using phenolphthalein (Sigma-Aldrich, Merck) as an indicator until the appearance of a pale pink color persisted for over 1 min. The results were calculated as the mean value of three successive experiments and expressed as the percent of malic acid. The pH values for each experimental group were measured by a pH-meter WTW pH 7110 (WTW, Weilheim, Germany) at 23°C (TUMBARSKI *et al.*, 2019).

2.2.3.2 Total phenolic content

The total phenolic content (TPC) was measured using a Folin-Ciocalteu reagent (Sigma-Aldrich, Merck) by the method of STINTZING *et al.* (2005). To conduct the analysis, 1 mL of Folin-Ciocalteu reagent was mixed with 0.2 mL blackberry juice and 0.8 mL 7.5% Na_2CO_3 (Sigma-Aldrich, Merck). The reaction was performed in darkness at room temperature for 20 min. The absorbance was measured by UV/Vis spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., Leeds, UK) against a blank at λ=765 nm, and the results were expressed as mg equivalent of gallic acid (GAE)/100 g of fresh weight (fw) according to a calibration curve (IVANOV *et al.*, 2014).

2.2.3.3 Total anthocyanins content

The total anthocyanins content (TAC) was estimated using the pH differential method (LEE *et al.*, 2005). The samples of blackberry juice (0.2 mL) were mixed with buffers (Sigma-Aldrich, Merck) at pH 1.0 and pH 4.5 (1.8 mL), and the absorbance was measured by UV/Vis spectrophotometer Camspec M107 (Spectronic-Camspec Ltd.) against a blank at λ=510 nm and λ=700 nm. The results were expressed as mg cyanidin-3-glycoside equivalents/100 g of fw.

2.2.3.4 Antioxidant activity

The antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging method. To perform this assay, 0.15 mL of blackberry juice was mixed with 2.85 mL of a freshly prepared 0.1 mM methanol solution of DPPH (Sigma-Aldrich, Merck). The samples were incubated in darkness for 15 min at 37°C. The reduction of absorbance was measured at λ=517 nm by UV/Vis spectrophotometer Camspec M107 (Spectronic-Camspec Ltd.) against a blank prepared with methanol (Sigma-Aldrich, Merck), and the percentage of inhibition was calculated (IVANOV *et al.*, 2014).

2.2.3.5 High pressure liquid chromatographic (HPLC) analysis of organic acids

The content of organic acids was determined by an Elite LaChrom HPLC-DAD system (VWR™ Hitachi) according to IVANOV *et al.* (2017). The separation was conducted on a Discovery® HS C18 column (5 μm, 25 cm × 4.6 mm) at 30°C. The isocratic elution was conducted with 25 mM KH_2PO_4 (pH 2.4) as a mobile phase at a flow rate of 0.5 mL/min. L-(+)-ascorbic acid was detected at λ=244 nm and L-malic acid at λ=210 nm. The obtained results were expressed as mg/100 g of fw.

2.2.3.6 HPLC analysis of mono- and disaccharides

Chromatographic separation and measurement of sugars were performed on an Elite LaChrom HPLC system coupled with a refractive index detector Chromaster 5450 (VWR™ Hitachi). The separation was performed on a Shodex® Sugar SP0810 column (300 mm × 8.0 mm) with Pb^{2+} and a guard column Shodex SP-G (5 μm, 6 mm × 50 mm) operating at 85°C mobile phase d.H_2O with a flow rate of 1.0 mL/min and injection volume of 20 μl. The results were expressed as g/100 g of fw (PETKOVA *et al.*, 2014).

2.2.4 Statistical analysis

Each analysis was independently replicated three times, the data were presented as mean value, and the standard deviation (±SD) was calculated (TUMBARSKI *et al.*, 2019).

3. RESULTS AND DISCUSSION

3.1. Visual observation

During the first four days of storage at 4°C and 75% RH, a slight increase in weight loss (WL) in all experimental groups was observed, which was greater in the control blackberries than in the pectin and pectin+bacteriocin coated fruit. During this time no morphological changes in any experimental group were observed (Figs. 1 and 2). On the 8-th day of the refrigerated storage, the first visible signs of dehydration in the control blackberries appeared, while the coated fruit remained unaffected (Fig. 3). The WL of the control blackberries was greater with 1.8% compared to that of the treated fruit. On the 12-

th day of the storage period, the first visible signs of fungal growth in the control and pectin-coated fruit appeared, while the bacteriocin-containing coatings prevented effectively the blackberries from decay and no spoilage changes were observed (Fig. 4). The WL of the control blackberries was greater with 2.1% (pectin) to 2.4% (pectin+bacteriocin) compared to the coated fruit. At the end of the observation period (day 16), the dehydration in the control group continued to increase, and the difference in the WL between the uncoated blackberries and the coated fruit reached 6.3% (pectin) and 6.7% (pectin+bacteriocin), respectively. The fungal spoilage process was most pronounced in the control and pectin-containing coatings, while the blackberries whose coatings contained a bacteriocin remained unaffected (Fig. 5).
As seen from the results summarized in Table 1, the pectin-based edible coatings singly or in combination with a bacteriocin of *B. methylotrophicus* BM47 effectively protected the treated fruit from moisture/weight loss and desiccation during the entire storage period, which helped to extend their storage life and to improve their commercial appearance. The protective effect of the bacteriocin of *B. methylotrophicus* BM47 and the attendant decrease in the decay percentage were related to the bacteriocin's strong antifungal properties as described in our previous research (TUMBARSKI *et al.*, 2018).
A similar tendency for weight loss during storage at low temperature was reported by GUERREIRO *et al.* (2015), who used citrus pectin and alginate as edible coatings for red raspberry fruit. In addition, it was demonstrated that pectin active coatings improved the physico-chemical, microbiological and sensory quality of strawberries from 6 (control) to 15 days at 4°C and 90% RH (VALDÉS *et al*., 2015).

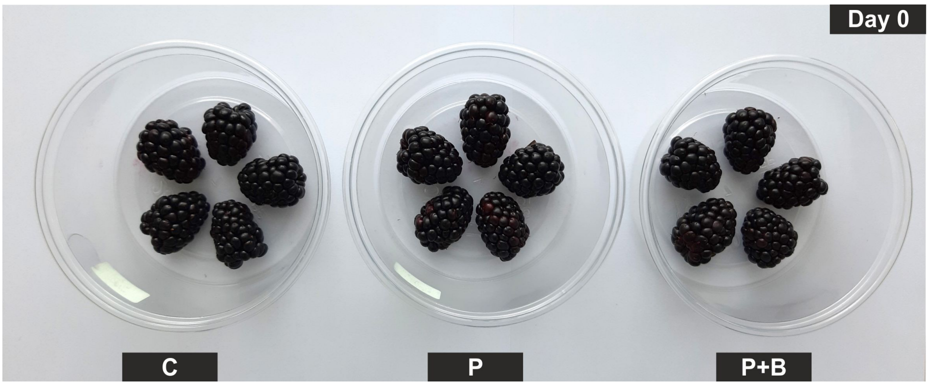

Figure 1. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on the appearance and morphology of fresh blackberries at the beginning of the experiment (day 0).
C - control; P - pectin (1%); P+B - pectin (1%) + bacteriocin.

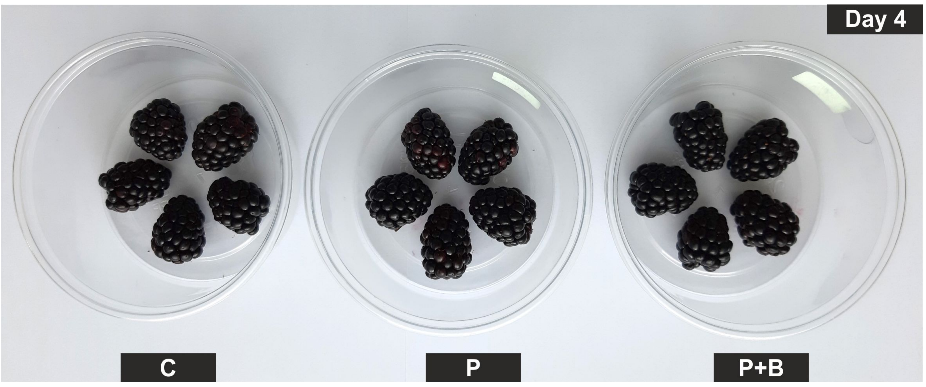

Figure 2. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on the appearance and morphology of fresh blackberries at day 4 during refrigerated storage (4°C and 75% RH). C - control; P - pectin (1%); P+B - pectin (1%) + bacteriocin.

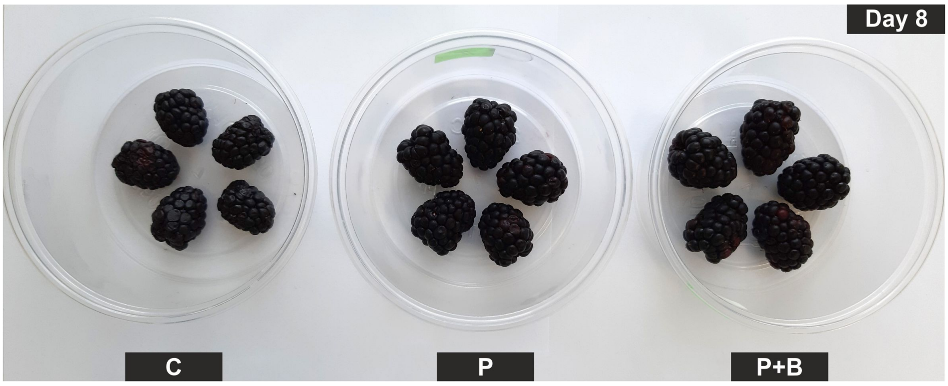

Figure 3. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on the appearance and morphology of fresh blackberries at day 8 during refrigerated storage (4°C and 75% RH). C – control; P - pectin (1%); P+B – pectin (1%) + bacteriocin.

Figure 4. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on the appearance and morphology of fresh blackberries at day 12 during refrigerated storage (4°C and 75% RH). C - control; P - pectin (1%); P+B - pectin (1%) + bacteriocin.

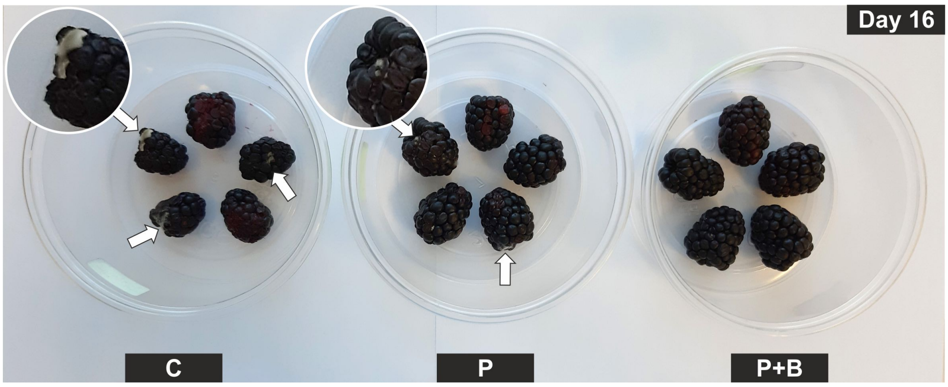

Figure 5. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on the appearance and morphology of fresh blackberries at day 16 during refrigerated storage (4°C and 75% RH). C - control; P - pectin (1%); P+B - pectin (1%) + bacteriocin.

3.2. Changes in total soluble solids, titratable acidity and pH

The total soluble solids maintained a constant level of 14.1% in all experimental groups during the first four days of storage. On the 8-th day, a slight increase in TSS in the control group and pectin-coated blackberries was detected, while pectin+bacteriocin coatings maintained TSS levels equal to the initial (14.1%). The TSS levels continued to rise gradually over the storage period due to water migration in the environment and fruit desiccation. By the end of the observation period (days 12 and 16) the treated blackberries had lower TSS values (with 0.2% to 0.4% for pectin-coated fruit and 0.4% to 0.6% for pectin+bacteriocin-coated fruit) compared to the uncoated blackberries (Table 1). These results demonstrated that pectin-based edible coatings, especially those with the addition of bacteriocin, provided a protective barrier against moisture loss and consequently a reduced attenuation of the fruit quality.

A slight increase in TA and a decrease in pH values in all groups were observed, both of which are normally associated with post-harvest changes of fruits. The results presented in Table 1 demonstrate that pectin and pectin+bacteriocin coatings did not consistently influence these two parameters, which remained similar to those observed in the uncoated blackberries until the end of the storage period. TOSUN *et al.* (2008), who studied the behaviour of pH during blackberry ripening, observed average values of 3.20 in unripe fruits, 2.64 in red fruits and 3.14 in mature fruits. According to ANTUNES *et al.* (2003), the initial values of pH 3.59 and 3.39 reached 3.94 and 4.09 after 12 days of storage, respectively; there was an increase in pH of 0.40 and 0.70. However, in our case, a decrease in pH values of 0.30 was observed at the end of 16 days.

In our study, an increase in titrable acidity was observed during the storage (Table 1) from 1.09 to 1.70 %. Our values were higher than reported from MENEGHEL *et al.* (2008), who showed constant values for acidity during 18 days of storage, with average values of 0.86, 0.89 and 0.85 g of citric acid per 100 g for coated blackberry cv. Comanche. The similar observation of increase in titrable acidity was reported by OLIVEIRA *et al.* (2012) in blackberry (*Rubus* spp.) conservation with edible coating.

Table 1. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on the physicochemical characteristics of fresh blackberries during refrigerated storage.

Day	Coating	Parameter				
		WL(%)	Decay(%)	TSS(%)	TA(%)	pH
0	C	n.a.	n.a.	14.1	1.09±0.06*	3.72±0.03
	P	n.a.	n.a.	14.1	1.08±0.03	3.71±0.06
	P+B	n.a.	n.a.	14.1	1.09±0.07	3.72±0.08
4	C	7.6	0	14.1	1.16±0.02	3.63±0.04
	P	6.7	0	14.1	1.19±0.04	3.62±0.02
	P+B	6.7	0	14.1	1.20±0.03	3.54±0.06
8	C	12.0	0	14.5	1.30±0.05	3.60±0.04
	P	10.2	0	14.3	1.41±0.01	3.56±0.03
	P+B	10.2	0	14.1	1.55±0.06	3.52±0.01
12	C	17.7	20.0	14.7	1.57±0.03	3.57±0.02
	P	15.6	10.0	14.5	1.61±0.01	3.45±0.04
	P+B	15.3	0	14.3	1.67±0.04	3.42±0.05
16	C	23.7	40.0	15.2	1.70±0.06	3.42±0.01
	P	17.4	20.0	14.8	1.72±0.02	3.46±0.05
	P+B	17.0	0	14.6	1.74±0.01	3.44±0.03

C - control; P - pectin (1%); B - bacteriocin; WL - weight loss; TSS - total soluble solids; TA - titratable acidity; n.a. - not analyzed; * - ±standard deviation (±SD).

3.3. Changes in organic acid content

The results showed that the content of ascorbic acid in blackberries declined in all treatments during the first 4 days of storage (Table 2). This finding was most likely associated with a stress reaction resulting from the effects of low temperatures on fruit metabolism. Thereafter, ascorbic acid concentrations gradually increased in all treatments relative to the storage period. The application of pectin-based coatings singly and in combination with bacteriocin of *B. methylotrophicus* BM47 effectively maintained higher levels of ascorbic acid in the treated fruit compared to the control fruit during the entire monitoring period. By the end of the storage period (16-th day), ascorbic acid levels had reached 56.8 mg/100 g of fw (control), 57.5 mg/100 g of fw (pectin) and 58.8 mg/100 g of fw (pectin+bacteriocin). These levels were close to the value noted at the beginning of the experiment (62.3 mg/100 g of fw). Our values for ascorbic acid content in fresh and coated blackberries were higher than reported values for different fresh blackberry cultivars (12.3–16.4 mg/100 g of fw) (DEIGHTON *et al*. 2000). The detected levels of ascorbic acid content were near to Cornelaian cherry cultivars (48.4-73.1 mg/100 g of fw) and higher than its level in other berries as raspberry (21.2- 31.1 mg/100 g of fw) and strawberries (46 mg/100 g of fw) (PANTELIDIS *et al*. 2007).

The concentration of malic acid in both the control and coated blackberries progressively increased, with storage time, reaching levels of 56.9 mg/100 g of fw (control), 58.3 mg/100 g of fw (pectin) and 61.3 mg/100 g of fw (pectin+bacteriocin) by the 16-th day of storage as compared to the initial level of 31.3 mg/100 g of fw. Our values for malic acid were more than twenty times higher than reported content in raspberry cultivars (ZORENC *et al*.

2017) and wild growing blackberries (MIKULIC-PETKOVSEK *et al.* 2012). Neither type of coating had an inhibitory effect on the increasing concentration of malic acid that is normally associated with post-harvest changes in fruits (Table 2). These results correlated with the increasing levels of titratable acidity and decreasing pH values in all experimental groups during the storage period (Table 1). This could be explained with by increased metabolism of fruits after harvesting, in which a greater consumption of organic acids is needed as substrates for the respiratory process. As a result of this, there is a greater conversion into simple sugars during maturation (OLIVEIRA *et al*. 2012).

Table 2. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on organic acids content and sugar content of fresh blackberries during refrigerated storage.

Day	Coating	Organic acid (mg/100 g)		Sugar (g/100 g)	
		Ascorbic	Malic	Glucose	Fructose
0	C	62.3±0.14*	31.3±0.22	3.0±0.21	3.4±0.22
	P	62.2±0.15	31.1±0.21	3.0±0.24	3.4±0.19
	P+B	62.1±0.13	31.2±0.17	3.0±0.18	3.4±0.23
4	C	47.5±0.16	31.3±0.14	n.a.	n.a.
	P	49.8±0.23	33.1±0.15	n.a.	n.a.
	P+B	51.8±0.21	31.3±0.23	n.a.	n.a.
8	C	53.4±0.19	33.2±0.24	3.0±0.29	3.4±0.27
	P	54.9±0.22	45.6±0.16	2.8±0.23	3.3±0.21
	P+B	55.2±0.18	46.2±0.13	2.7±0.25	3.4±0.28
12	C	56.0±0.15	47.7±0.15	n.a.	n.a.
	P	56.4±0.17	56.1±0.18	n.a.	n.a.
	P+B	58.2±0.21	56.2±0.22	n.a.	n.a.
16	C	56.8±0.24	56.9±0.21	2.8±0.25	3.2±0.28
	P	57.5±0.15	58.3±0.19	2.5±0.22	3.1±0.24
	P+B	58.8±0.21	61.3±0.15	2.3±0.29	3.2±0.23

C - control; P - pectin (1%); B – bacteriocin; n.a. - not analyzed; * - ±standard deviation (±SD).

3.4. Changes in sugar content

The values of glucose and fructose in blackberries were measured at the beginning (day 0), middle (day 8) and end (day 16) of the storage period (Table 2). The detected sugars in all samples were only glucose and fructose as their content was closer to reported values for wild grown blackberry 35g/kg of fw (MIKULIC-PETKOVSEK *et al*. 2012). However, in our study sucrose was not found in blackberry samples. The results showed that on the 8-th day of the experiment, the concentration of glucose in the control fruit matched the initial level of 3.0 g/100 g of fw, while the glucose level in the treated fruit decreased slightly to 2.8 g/100 g of fw (pectin) and 2.7 g/100 g of fw (pectin+bacteriocin). Thereafter, the concentration of glucose decreased in all treatments and reached levels of 2.8 g/100 g of fw (control), 2.5 g/100 g of fw (pectin) and 2.3 g/100 g of fw (pectin+bacteriocin) by the 16-th day of storage. Fructose levels remained almost constant despite prolongation of the storage time. During the first 8 days of storage, all treatments maintained their initial level of 3.4 g/100 g of fw, except the pectin-coated fruits, which showed a concentration of 3.3 g/100 g of fw. At the end of the storage period (day 16), the concentration of fructose had decreased slightly in all experimental groups, reaching levels of 3.2 g/100 g of fw (control), 3.1 g/100 g of fw (pectin) and 3.2 g/100 g of fw (pectin+bacteriocin) (Table 2). These results demonstrated that pectin-based edible coatings singly or in combination with bacteriocin did not protect the sugar content in coated blackberries under refrigerated storage conditions.

3.5. Changes in total anthocyanins content

As seen in Fig. 6, the initial level of total anthocyanins of 109.2 mg/100 g of fw was in accordance with that reported in the literature (104 – 198 mg/100 g of fw) (PANTELIDIS *et al.*, 2007), but gradually decreased in all treatments. This trend was recorded through the end of the observation period. By the 16-th day of storage, total anthocyanins reached concentrations of 100 mg/100 g of fw (control), 94.2 mg/100 g of fw (pectin) and 92.3 mg/100 g of fw (pectin+bacteriocin), indicating that the application of both coatings failed to delay the reduction of anthocyanins in blackberries during refrigerated storage. The negative effect of low temperatures on anthocyanin concentration during the storage of fresh berries was previously reported by KALT *et al*. (1999). Lowered anthocyanins and ascorbic acid content during cold storage of strawberries was also confirmed by CORDENUNSI *et al.* (2005). However, the authors observed a positive impact on other parameters such as sugars, while levels of flavonols, ellagic acid, TPC and antioxidant activity remained almost the same or even decreased at all tested temperatures (6, 16 and 25°C).

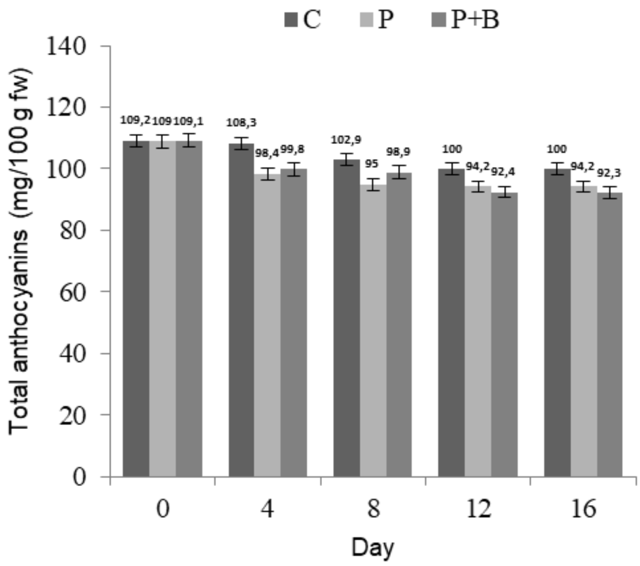

Figure 6. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on the total anthocyanins content of fresh blackberries during refrigerated storage. C - control; P - pectin (1%); P+B - pectin (1%) + bacteriocin.

3.6. Changes in total polyphenols content

During the first four days of refrigerated storage, the TPC in both the control and treated blackberries maintained relatively high levels that were close to the initial concentration of polyphenols of 186 mg GAE/100 g of fw (0 day) (Fig. 7). However, by the end of the observation period (16-th day), the TPC declined for all experimental groups to concentrations of 174.2 mg GAE/100 g of fw (control), 173.8 mg GAE/100 g of fw (pectin) and 173.1 mg GAE/100 g of fw (pectin+bacteriocin). These results demonstrated that the coatings did not effectively protect the polyphenolic content, and this progressive decrease was most likely associated with the breakdown of cell structures that is caused by natural senescence processes in the fruits (TUMBARSKI *et al.*, 2019).

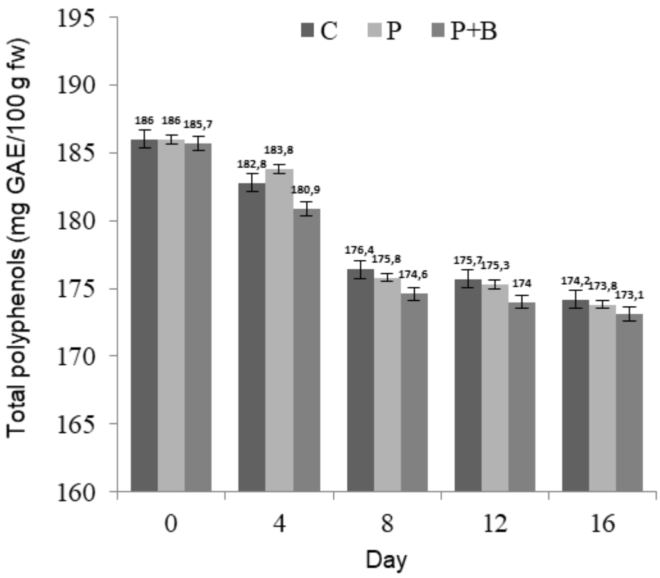

Figure 7. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on the total phenolic content of fresh blackberries during refrigerated storage. C - control; P - pectin (1%); P+B - pectin (1%) + bacteriocin.

3.7. Changes in antioxidant activity

The results obtained by the DPPH method showed that the antioxidant activity of control blackberries gradually decreased throughout the monitoring period, reaching its lowest value of 222.2 mmol TE/100 g of fw on the 16-th day. As seen from the results presented in Fig. 8, the pectin and pectin+bacteriocin coatings effectively inhibited this decrease and maintained antioxidant levels in the treated blackberries that were higher than those in the uncoated ones, keeping the antioxidant levels close to the initial value of 233.9 mmol TE/100 g of fw (0 day). The positive impact of pectin and pectin+bacteriocin coatings on antioxidant activity could be associated with a reduction in the respiration rates and the resultant protective effect on other biologically active compounds that possess strong antioxidant activity such as phenolic acids, hydrolysable tannins, vitamin E, carotenoids, minerals and enzymes. Further exploration of these antioxidant compounds is warranted in a future study.

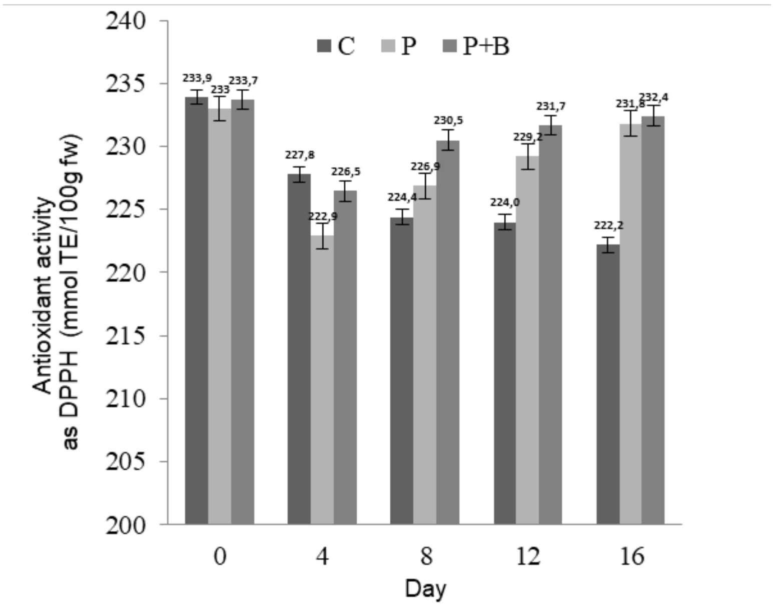

Figure 8. Effects of pectin-based and pectin-based edible coatings with bacteriocin of *B. methylotrophicus* BM47 on the antioxidant activity of fresh blackberries during refrigerated storage. C - control; P - pectin (1%); P+B – pectin (1%) + bacteriocin.

The biopreservation of blackberry fruit using different edible coatings has also been investigated by other authors. For example, OLIVEIRA *et al.* (2012) reported the effects of three types of edible coatings - chitosan (1.5%), cassava starch (2.5%) and kefir grains in water (20%) on the storage life of the blackberry cultivar *Tupy* stored at temperatures of 0°C and 10°C. The authors stated that during the 18 days of storage, all coatings effectively reduced the loss of weight at 0°C, but cassava starch and kefir grains-based coatings were not efficient at 10°C. During storage, a significant increase in pH was observed in all treatments at both 0°C and 10°C, with the control fruit showing the lowest pH values. The authors also reported that at 10°C, the anthocyanins levels in all treatments progressively decreased until the 12-th day but thereafter increased significantly in the control fruit and in blackberries with chitosan coatings. At 0°C, the same trend of lowered anthocyanins concentrations in the control and chitosan coatings was observed, while cassava starch and kefir grains-based edible coatings maintained elevated and relatively stable anthocyanins levels.

PÉREZ-GALLARDO *et al.* (2014) developed more hydrophobic edible coatings based on modified tapioca starch added to 0.5 and 1.0% beeswax microparticles and then examined the quality of freshly harvested blackberries during storage at 4°C for 16 days. This study showed that the coated fruit exhibited greater weight loss than uncoated fruit, but the increase in concentration of beeswax particles from 0.5 to 1% led to a decrease in weight loss of 11.55±0.71 and 9.72±0.42%, respectively, compared to the control fruit (7.6±0.13%) at the end of the experiment. Uncoated blackberries showed higher anthocyanins content than coated ones. The coated blackberries with 0.5% beeswax microparticles exhibited low but similar TAC, while those coated with 1% beeswax microparticles revealed higher values and a slight, but significant decrease at the end of the storage period. By the end of the storage period, the TPC was significantly higher in uncoated blackberries than in coated fruit.

GOL *et al*. (2015) evaluated the effects of edible coatings comprised of chitosan, alginate and carboxymethyl cellulose on the improvement in the quality and storage life of Indian blackberry or Jamun fruit (*Syzygium cumini* L.). The results from this study showed that fruit treated with these three coatings at concentrations of 1% and 1.5% exhibited a significant delay in WL along with a reduction in decay percentage as well as positive effects on TSS, pH, TA and sugars in comparison to the uncoated fruit. These coatings also had a positive impact on maintaining a higher concentration of antioxidants during the 16 days of storage and had a positive effect on the inhibition of cell wall-degrading enzyme activities.

4. CONCLUSIONS

The present study demonstrated that the application of celery pectin-based edible coatings singly and in combination with a bacteriocin of *Bacillus methylotrophicus* BM47 represents a promising approach for extending storage life in processed blackberries. The bacteriocin-containing edible coatings effectively inhibited the fungal growth, significantly reduced the decay incidence, delayed the increase in TSS and helped to preserve some of the health benefitial properties of the fresh fruits, specifically the ascorbic acid content and antioxidant activity. Our results suggested that the bacteriocin synthesized by *B. methylotrophicus* BM47 in the composition of edible coatings could find a successful application as a biopreservative for improving product quality, storage life and the safety of blackberry fruit.

ACKNOWLEDGEMENTS

This work was financed by Fund 'Science' of the University of Food Technologies, Plovdiv, Bulgaria (Grant 01/19-H). The authors declare no conflict of interest.

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Paper Received August 15, 2019 Accepted January 31, 2020