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Biopreservation of Fresh Strawberries by Carboxymethyl Cellulose Edible Coatings Enriched with a Bacteriocin from *Bacillus methylotrophicus* BM47

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SUMMARY

Bacteriocins are a large group of antimicrobial compounds that are synthesized by representatives of the genus *Bacillus* and lactic acid bacteria (LAB). Bacteriocins are used extensively in the food industry as biopreservatives. Incorporated in the composition of edible coatings, bacteriocins can reduce microbial growth and decay incidence in perishable fruits, thus improving product shelf-life and commercial appearance. The present study aims to investigate the effect of edible coatings of 0.5 % carboxymethyl cellulose (CMC) enriched with a purified bacteriocin from *Bacillus methylotrophicus* BM47 on the shelf-life extension of fresh strawberries. During storage at 4 °C and 75 % relative humidity (RH) for 16 days, measurements of mass loss, decay percentage, total soluble solids (TSS), titratable acidity (TA), pH, organic acids, total phenolic and anthocyanins contents and antioxidant activity were taken. The results demonstrated that the application of 0.5 % CMC and 0.5 % CMC+bacteriocin (CMC+B) edible coatings led to a significant decrease of mass loss in treated strawberries compared to the uncoated fruit. After the 8-th day of storage, significant reductions in decay percentage along with the absence of fungal growth in CMC+B-coated fruit were observed in comparison to the CMC-coated and control strawberries.

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During the second half of the storage period, CMC and CMC+B treatments reduced TSS levels in the coated fruit compared to the control, but did not affect increased TA and lowered pH values that are normally associated with post-harvest changes. The CMC and CMC+B coatings were ineffective against the decrease in ascorbic acid, total phenolics and anthocyanins content during cold storage. The application of CMC and CMC+B coatings exhibited a significant inhibitory effect on decreasing antioxidant activity throughout the storage period and maintained the antioxidant levels in both treatments close to the initial value of 76.8 mmol TE/100 g of fm.

Key words: bacteriocin, biopreservation, edible coatings, strawberry, *Bacillus methylotrophicus*

INTRODUCTION

The cultivated or garden strawberry (*Fragaria* × *ananassa* Duch.) is among the most wide spread of fruits and is commonly used as a dessert in the human diet. Strawberry fruit in its fresh or processed form has a delicious taste and unique flavor that is widely accepted by consumers. Strawberries are also very nutritious and exert a strong antioxidant activity that is related to the high levels of anthocyanins, phenolic compounds, flavonoids, nitrogenous compounds, tocopherols, carotenoids and ascorbic acid. In addition to antioxidants, strawberry fruit contains a variety of volatiles such as esters, aldehydes, alcohols and sulfur compounds. The rich phytochemical composition and high antioxidant capacity of strawberries exhibit a protective effect against chronic and degenerative diseases, cardiovascular disorders, and have been found to possess anticarcinogenic and antimutagenic activities (*1,2*).

Strawberries are extremely sensitive and perishable. They have a short shelf-life due to exposure to post-harvest activities that make the strawberries susceptible to physical injuries, fungal decay, desiccation and other disorders during storage (*3*). Despite the use of rapid cooling to avoid post-harvest decay and to maintain the quality of the fruit during the storage, the shelf-life of fresh strawberries at low temperatures (0–4 °C) is limited to 5 days (*4*).

In recent years, more advanced trends in food biopreservation have resulted in the use of non-conventional approaches such as the application of edible coatings that help to extend the shelf-life of strawberries and other perishable fruits. Edible coatings are thin layers of edible materials that are applied to the surface of food products and play an important role in product conservation. The basic function of edible coatings is to protect the product from mechanical damage, physical, chemical and microbiological activities (*5*). The edible coatings act as protective

barriers against dehydration by reducing water/moisture loss, which is the primary reason for deterioration of fruit quality. In addition to decreasing in the loss of product mass and firmness, edible coatings suppress respiration, improve textural quality, help to retain natural color and volatile flavor compounds, and retard microbial growth especially fungal growth (*4,6*).

The effectiveness of edible coatings depends on the selection of coating materials, which can be used alone or in various combinations. Numerous studies concerning the composition and application of edible coatings have shown that polysaccharides, proteins, lipids or their derivatives are among the most commonly used structural matrices (*7*). For example, cellulose is a suitable natural polysaccharide of plant origin used in the production of edible coatings. To overcome the limitations associated with the native form of this biopolymer (water-insolubility and highly associated crystalline structure), some commercially available derivatives such as carboxymethyl cellulose (CMC), methyl cellulose (MC), hydroxyl propyl cellulose (HPC) and hydroxyl propyl methyl cellulose (HPMC) have been developed for incorporation into edible coatings used for perishable fruits (*8*). Cellulose derivatives are water-soluble, odorless and tasteless, possess good film-forming characteristics and provide a moderate barrier to moisture and oxygen transmission (*9*). In addition to its edible coating application, carboxymethyl cellulose is considered to be a promising raw material to replace non-degradable polymers in the packaging of fruits and vegetables (*10*).

One of the most challenging problems associated with the shelf-life, storage and distribution of perishable fruits, and especially strawberries, is fungal spoilage. Recently, the increasing consumer demand for fresh and minimally processed food has resulted in research into the development and application of new methods for biopreservation by safe and non-toxic biologically active compounds as alternatives to traditional chemical treatments and fungicides. In this regard, bacteriocins as antimicrobial peptides produced by some *Bacillus* spp. and lactic acid bacteria (LAB) represent a promising solution to problems associated with fungal decay. A number of recent studies have reported the potential of LAB bacteriocins (nisin, bovicin CH5, enterocins AS-48 and 416K1, bificin C6165 and pediocin) in the biopreservation of fruits and fruit products (*11,12*). Some other LAB bacteriocins such as those produced by *Lactobacillus plantarum* and *Lactobacillus* Y153 have been shown to possess a strong inhibitory effect on the most severe etiological agents of post-harvest fungal decay in strawberries - *Rhizopus stolonifer* and *Botrytis cinerea* (*13,14*). However, there are no data available in the scientific literature related to the application of other *Bacillus* sp. bacteriocins in fruit biopreservation.

Therefore, the purpose of the present study was to investigate the application of a bacteriocin produced by *Bacillus methylotrophicus* BM47 to the composition of carboxymethyl cellulose edible coatings and to evaluate the effect of bacteriocin treatment on the prolongation of shelf-life and the preservation of quality in fresh strawberries.

MATERIALS AND METHODS

Fruit material

Fresh strawberries (*Fragaria* × *ananassa* Duch.) were purchased from a local fruit market in Plovdiv, Bulgaria. The strawberries were selected by uniform size, normal shape without defects or physical damage and saturated red color. Fruits were kept in brown paper bags and transferred immediately to the laboratory to conduct the experimental work.

Bacteriocin

A bacteriocin produced by *Bacillus methylotrophicus* strain BM47 isolated from a natural thermal spring in the Haskovo region of Bulgaria was used. The purified bacteriocin substance contained an antimicrobial peptide of intermediate molecular size (19 578 Da) as described in our previous work (*15*).

Preparation of edible coatings

An edible coating solution was prepared according to the method described by Gol *et al.* (*16*) with slight modification. Carboxymethyl cellulose - CMC (0.5 %) was prepared by dissolving 2 g of CMC powder (Fooding, Shanghai, China) in a 400 mL distilled water-ethyl alcohol mixture (3:1 L/L) at 75 °C while being stirred by a magnetic stirrer IKA® RCT classic (IKA®-Werke GmbH & Co. KG, Staufen, Germany) for 20 min at 800 rpm. Next, 0.25 % glycerol monostearate Cutina® GMS (Henkel, Düsseldorf, Germany) was added to the mixture, and the solution was stirred under the same conditions for 15 min. Ethyl alcohol (Sigma-Aldrich, Merck, St. Louis, MO, USA) in the solution was used to reduce drying time and to obtain transparent and shiny coatings, while GMS was used as a plasticizer to improve the flexibility and strength of the coatings. The coating solution was divided into two portions of 200 mL each. After cooling, 100 AU/mL (0.15 mg/mL) of purified and lyophilized bacteriocin substance was added to the second portion and stirred without heating for 15 min at 800 rpm.

Application of edible coatings

Strawberries were surface-disinfected by dipping in 1 % sodium hypochlorite (Sigma-Aldrich, Merck) for 3 min, then washed three times and dried at 25 °C for 2 h in a drying chamber with forced air MLW (Labexchange, Burladingen, Germany). The strawberries were divided into three experiment groups of 25 strawberries each with uncoated strawberries labeled as the control (group 1), strawberries with a CMC coating (group 2) and strawberries with a CMC coating+bacteriocin (CMC+B or group 3). After drying, the strawberries from groups 2 and 3 were dipped into the relevant coating solutions for 2-3 min. The processed fruit was then dried at 25 °C for 2 h in a drying chamber with forced air MLW (Labexchange). Next, the strawberries from all groups were placed into plastic boxes and stored at 4 °C and 75 % relative humidity (RH) for 16 days. The control fruit were examined at the beginning of the experiment (*i.e*. 0 day). During the period of storage, all groups were observed for morphological changes and fungal growth on the 4-th, 8-th, 12-th and 16-th day, and samples for physicochemical analyses were collected (*16*).

Mass loss percentage and decay percentage

To estimate these two parameters, three separate groups consisting of ten strawberries with the same treatments (control, CMC and CMC+B) were prepared and stored under the same conditions. To determine the mass loss percentage, 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 the storage period. The difference between the initial mass of each experiment group and the mass of the same group determined on each sampling day was defined as a mass loss, and the results were calculated as a percentage loss of the initial mass using the following equation:

$$\text{Mass loss}(\%) = \frac{M_0 - M_1}{M_0} \times 100$$

M_0 is the initial mass of each experiment group (day 0), and M_1 is the measured mass of the same group on each sampling day (*17*).

The decay percentage was determined as follows: the number of strawberries with visible decay or morphological changes was expressed as % of the initial number of all strawberries in the relevant experiment group (*16*).

Total soluble solids, titratable acidity and pH

The total soluble solids (TSS) of the strawberries was measured using a portable Abbe refractrometer (Officine Galileo, Campi Bisenzio, Italy). Strawberries from each experiment group

were homogenized with a homogenizer Polytron (Kinematica AG, Luzern, Switzerland). A few drops of the juice were placed on the prism glass, and the TSS value was read directly and recorded. The titratable acidity (TA) was determined by titration of 2 mL of strawberry juice with 0.1 M NaOH (Sigma-Aldrich, Merck) using phenolphthalein (Sigma-Aldrich, Merck) as an indicator until the appearance of a pale pink color persisting over 1 min. The results were calculated as the mean value of three consecutive experiments and expressed as the percent of citric acid. The pH values of each group were measured by a pH-meter WTW pH 7110 (InoLab®, Weilheim, Germany) at room temperature (23 °C) (*16*).

Total phenolic content

Total phenolic content (TPC) was measured by the method of Stintzing *et al.* (*18*). In brief, 1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, Merck) diluted five times was mixed with a 0.2 mL sample and 0.8 mL 7.5 % Na_2CO_3 (Sigma-Aldrich, Merck). The reaction was performed in darkness at room temperature for 20 min. Next, the absorbance was measured by UV/VIS spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., Leeds, United Kingdom) at λ=765 nm against a blank. The results were expressed as mg equivalent of gallic acid (GAE) per 100 g of fresh mass (fm), according to a calibration curve (*19*).

Total anthocyanins content

Total anthocyanins content was determined according to the pH differential method, described by Lee *et al.* (*20*). Samples from the strawberry juice (0.2 mL) were mixed with buffers at pH=1.0 and pH=4.5 (1.8 mL), and the absorbance was measured against a blank at λ=510 nm and λ=700 nm. The results were expressed as mg cyanidin-3-glycoside equivalents/100 g of fm for a minimum of three replicates.

Antioxidant activity

The antioxidant activity was determined by ferric reducing antioxidant power (FRAP) assay according to the Benzie and Strain method (*21*) with slight modification. The FRAP reagent was freshly prepared by mixing 10 parts 0.3 M acetate buffer (pH=3.6), 1 part 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3.6H_2O$ (all from Sigma-Aldrich, Merck) in distilled water. The reaction was started by mixing 3.0 mL FRAP reagent with 0.1 mL of strawberry juice. The reaction time was 10 min at 37°C in darkness, and the absorbance was measured at

λ=593 nm against a blank prepared with methanol. Antioxidant activity was expressed as mmol Trolox® equivalents (TE) per 100 g of fm (*19*).

High pressure liquid chromatographic (HPLC) analysis of organic acids

The organic acids analysis was performed on an Elite LaChrom HPLC-DAD system (VWR™ Hitachi, Tokyo, Japan) as previously described by Ivanov *et al.* (*22*). The separation was conducted on a Discovery® HS C18 column (25 cm×4.6 mm, 5 µm) (Sigma-Aldrich, Merck) at 30 °C by isocratic elution with the mobile phase consisting of 25 mM KH_2PO_4 (pH=2.4 with 85 % H_3PO_4) at a flow rate of 0.5 mL/min. The detection of L-(+)-ascorbic acid (vitamin C) was monitored at λ=244 nm and for citric and L-malic acids at λ=210 nm, respectively. The results were expressed in mg/100 g of fm.

Statistical analysis

The experiments were performed in triplicates, data were presented as mean value, and the standard deviation (±SD) was calculated.

RESULTS AND DISCUSSION

Mass loss percentage and decay percentage

The results from determination of the mass loss percentage are presented in Table 1. During the first four days of storage at 4 °C and 75 % RH, a slight increase in mass loss was observed, which was more pronounced in the control fruit than in the strawberries with CMC and CMC+B coatings. The same trend was recorded on the 8-th and 12-th days of the observation period - the mass loss of uncoated strawberries was higher compared to that of the coated fruit and this parameter was lower with 1.22 % to 1.88 % (for CMC coatings) and 1.28 % to 1.84 % (for CMC+B coatings). The mass loss continued to increase, and by the end of storage period (16-th day) the difference between the control fruit and treated groups (CMC and CMC+B) reached 2.91 % and 2.96 % respectively, which demonstrated that these coatings prevented moisture/water loss in the treated fruit.

Our data also demonstrated that no morphological changes were observed in any of the experiment groups until the 4-th day of storage (Table 1). The first visible signs of fungal decay in

the control and CMC-coated fruit appeared on the 8-th day of the storage, while the strawberries with CMC+B coatings remained unaffected. Through the end of the storage period, a high reduction in decay percentage and no fungal growth in CMC+B-coated fruit were noted. This result would suggest that the preventive effect of the bacteriocin of *B. methylotrophicus* BM47 and the concomitant decrease in the incidence of mold decay in the CMC+B-covered strawberries were related to the antifungal activity of the bacteriocin as described in our earlier studies (*23*).

Our results were in agreement with those reported by Gol *et al.* (*16*) who described the effectiveness of 1 % CMC edible coatings in strawberries, showing a reduction in mass loss from 2.28 % on the 4-th day to 9.38 % on the 12-th day and higher decay percentage varying from 5.89 % on the 4-th day to 28.57 % on the 12-th day of storage. A significant delay effect in CMC-based coatings on the mass loss in fresh strawberries was also reported by Dong and Wang (*24*). Those authors found a lower mass loss of 16.23 % and a decay percentage of 71.8 % in the treated fruit, compared to the control fruit levels of 24.73 % and 93.2 %, respectively, on the 6-th day of storage.

Hussain *et al.* (*25*) reported that the application of CMC coatings (0.5 % and 0.75 %) alone had no effect on delaying decay or inhibiting fungal growth in strawberry fruit. However, compared with other treatments, the combination of 1 % CMC coatings with gamma irradiation (2.0 kGy) had a significant effect on delaying the decay and appearance of fungal growth in strawberries kept in refrigerated storage up to 18 days.

Changes in total soluble solids, titratable acidity and pH

In the control group only, a slight increase in total soluble solids (TSS) was detected on the 8-th day of storage. This change was related to the migration of water in the environment and the higher moisture loss of the uncoated fruit. The TSS levels in CMC-coated and CMC+B-coated strawberries were equal to the levels noted at the beginning of the experiment (8.4 %). The TSS continued to increase gradually, and the value of the uncoated group by the end of the storage period was higher with 0.5 % compared to the CMC-coated and CMC+B-coated fruit (Table 1).

A slight increase in titratable acidity (TA) and a decrease in pH values were detected in all experiment groups. These findings are usually associated with post-harvest physiological activities such as the decomposition of sugars and decay changes. The results summarized in Table 1 show that the application of CMC coatings and CMC+B coatings did not consistently influence these two parameters which remained similar to those of the control samples until the end of the storage period.

The changes in TSS were in agreement with the results published by Gol *et al.* (*16*) who also found that TSS levels increased during the storage period in both CMC-coated and uncoated strawberries. In contrast to our results, the authors reported a decrease in TA levels and an increase in pH values during the entire storage period.

According to Hussain *et al.* (*25*) the combination treatment of CMC coatings (1 %) and gamma irradiation (2.0 kGy) was helpful in maintaining higher levels of TSS in strawberries compared with the 0.5 % and 0.75 % CMC treatments and controls. The authors also noted decreasing TA levels in all treatments up to the 15-th day followed by an increase in TA levels after the 18-th day of the storage period.

Table 1. …..

Changes in organic acid content

The results obtained from the HPLC analysis of organic acids demonstrated that the content of ascorbic acid (vitamin C) in the strawberries gradually decreased in all experiment groups with prolongation of the storage period (Table 2). During the first 4 days, vitamin C levels in the three experiment groups decreased but were close to the initial level of 30.5 mg/100 g of fm (*i.e.* 0 day). Vitamin C concentrations continued to decline, and by the end of the monitoring period (16-th day) they reached levels of 17 mg/100 g of fm (control), 16 mg/100 g of fm (CMC coatings) and 15.7 mg/100 g of fm (CMC+B coatings). One explanation for the reduction of ascorbic acid content in strawberry fruit is the high thermal instability of ascorbic acid and the activity of ascorbate oxidase which converts ascorbic acid into dehydroascorbic acid with prolongation of the storage time (*2*).

Table 2. .....

Treatment with other biologically active substances could have a protective effect on ascorbic acid retention. Jia *et al.* (*13*) reported a post-harvest decrease in vitamin C in fresh strawberries. However, the authors observed a significantly higher content of vitamin C in fruit treated with bacteriocin of *Lactobacillus plantarum* LF-1 alone, compared to the control and nisin-treated fruit. After six days of storage, the bacteriocin LF-1-treated fruit showed a vitamin C content of 63 mg/100 g, while the nisin-treated fruit exhibited 50 mg/100 g. Strawberries treated with bacteriocin LF-1 had a 33 mg/100 g higher vitamin C level than that of the untreated fruit. Zhou *et al.* (*14*) reported that the application of a bacteriocin of *Lactobacillus* Y153 had an inhibitory effect on the progressive decrease of vitamin C in fresh strawberries, thus preserving the nutritional

qualities of the fruit. Similar results were obtained by Gol *et al.* (*16*) who concluded that edible coatings made up of CMC (1 %) with or without the addition of chitosan (1 %) exerted a significant effect on the level of ascorbic acid and delayed the decrease of ascorbic acid in treated strawberries.

The edible coatings of CMC and CMC+B did not affect the malic acid content of the strawberries during the storage period (Fig. 1). The malic acid content remained relatively constant during the first half of the storage period in comparison to the initial level of 22 mg/100 g of fm. However, by the end of the storage period (16-th day), malic acid content had decreased to values of 17 mg/100 g of fm (control), 16 mg/100 g of fm (CMC-coated strawberries) and 15.7 mg/100 g of fm (CMC+B-coated strawberries).

The concentration of citric acid, one of the primary acids in strawberries, gradually increased in both the control and coated fruit over the storage period, reaching levels of 69.8 mg/100 g of fm (control), 71.7 mg/100 g of fm (CMC) and 71.8 mg/100 g of fm (CMC+B) on the 16-th day of storage, compared to its initial concentration of 54.5 mg/100 g of fm (Fig. 1). The CMC and CMC+B edible coatings had no inhibitory effect on the increasing concentration of citric acid that is normally associated with post-harvest changes in the fruit. These results corresponded to the increasing titratable acidity and decreasing pH values (Table 1). Our results were in agreement with those reported by Nunes *et al.* (*26*) who observed increasing levels of citric acid in strawberries stored for 8 days under refrigerated conditions (1 °C and 90–95 % RH).

Changes in total anthocyanins content

Anthocyanins, a type of flavonoid, are the pigments that give strawberries their characteristic red color. The anthocyanins showed relatively constant levels in both uncoated and coated fruit during the first half of the storage period. The total anthocyanins retained concentrations of 14.55 mg/100 g of fm (control), 14.16 mg/100 g of fm (CMC) and 14.33 mg/100 g of fm (CMC+B), which were close to the initial levels of 14.91 mg/100 g of fm. Thereafter, the total anthocyanins began to decrease slightly. This trend was observed until the end of monitoring period when total anthocyanins reached levels of 10.98 mg/100 g of fm, 10.9 mg/100 g of fm and 10.82 mg/100 g of fm respectively, demonstrating that CMC and CMC+B coatings did not delay the reduction of anthocyanins content in strawberries during storage (Fig. 1).

Figure 1. .....

One of the explanations for decreasing anthocyanins and vitamin C levels in strawberries is the negative effect of low temperatures on their accumulation levels during cold storage as reported by Cordenunsi *et al.* (*27*). However, the authors observed a positive effect on soluble sugars, while flavonols, ellagic acid, total phenolic content and antioxidant activity maintained consistent levels or even decreased at all storage temperatures (6, 16 and 25 °C). The negative effect of low temperatures on anthocyanin biosynthesis during the cold storage of small fruits (*i.e.* strawberries) was also proven by Kalt *et al.* (*28*). A declining trend in anthocyanins content was likewise observed by Hussain *et al.* (*25*), who reported that strawberries treated with a combination of CMC edible coatings (0.5–1.0 %) and gamma irradiation (2.0 kGy) exhibited an initial increase in anthocyanins levels, followed by a sustainable decrease in both the control and treated fruit after prolonged storage.

According to some research, the addition of essential oils in the composition of CMC-based edible coatings could prevent the decrease in anthocyanins content. Dong and Wang (*24*) ascertained that strawberries coated with CMC (1 %) showed a reduction in anthocyanins content during storage. However, the authors found that the addition of garlic essential oil – GEO (2 %) into the CMC coatings was an effective way to delay fruit senescence and the decrease in anthocyanins. The authors concluded that CMC+GEO coatings acted as a gas barrier and modified the internal atmosphere of the fruit and in this way retarded the biochemical reactions leading to anthocyanin synthesis.

Changes in total polyphenols content

The total polyphenols maintained relatively high and almost equal concentrations in all experiment groups during the entire storage time, corresponding to the results obtained for total anthocyanins. Until the 8-th day, the total polyphenols level remained stable, and a slight decrease in their concentration was recorded on the 12-th day of observation period. On the 16-th day, the total polyphenols level decreased to 8.66 mg GAE/100 g of fm (uncoated fruit), 8.92 mg GAE/100 g of fm (CMC) and 8.69 mg GAE/100 g of fm (CMC+B) compared to the initial concentration of 12.25 mg GAE/100 g of fm (Fig. 2).

Figure 2. .....

Our results were in agreement with those reported by Gol *et al.* (*16*) who also observed a progressive decrease in total phenols in both the control and CMC (1 %) coated strawberries. However, the incorporation of chitosan (1 %) in these coatings helped to maintain higher phenolic

content in the processed fruit. The noted decrease of phenolic compounds, which might be associated with the breakdown of cell structures caused by natural fruit senescence during storage, was also stated by Dong and Wang (*24*) who determined that the control fruit had a greater phenolic decrease than fruit treated with CMC+GEO composite coatings.

The use of non-conventional methods in combination with edible coatings may also have a beneficial effect on the phenolic compounds in strawberries. The combined application of CMC edible coatings (1.0 %) and gamma irradiation (2.0 kGy) led to a significant increase in the total phenols in treated strawberries during 21 days of storage compared with the phenolic levels in fruit coated with 0.5 %, 0.75 % and 1 % CMC alone as reported by Hussain *et al.* (*25*).

Changes in antioxidant activity

The results obtained by FRAP assay (Fig. 3) revealed that the antioxidant activity of uncoated strawberries gradually decreased throughout the storage period, reaching its lowest value of 50.86 mmol TE/100 g of fm in the control fruit on the 16-th day. This result correlated with decreasing levels of compounds with antioxidant activity – ascorbic acid, anthocyanins and polyphenols. The application of CMC and CMC+B edible coatings inhibited this decrease and maintained the antioxidant activity of the coated fruit at levels higher than those of the uncoated ones and kept the antioxidant values close to the initial one of 76.8 mmol TE/100 g of fm (0 day). The protective effect of the edible coatings on antioxidant activity could be associated with a reduction in the respiration rate of the coated fruit and a probable positive effect on other biologically active compounds possessing antioxidant activity (phenolic acids, hydrolysable tannins, vitamin E, carotenoids, minerals, enzymes, etc.), which may be explored in a further study.

Figure 3. …..

CONCLUSIONS

Our results showed that the application of CMC and CMC+B edible coatings had a positive impact on some physicochemical parameters of strawberries by reducing the TSS levels and mass loss percentage. The addition of bacteriocin of *B. methylotrophicus* BM47 in the composition of CMC-based coatings (CMC+B) led to a significant decrease in decay incidence and effectively inhibited the fungal growth in the coated fruit. CMC and CMC+B edible coatings positively influenced one of the primary health benefit properties of fresh strawberries - their antioxidant activity. Thus, we can conclude that CMC-based edible coatings in combination with the bacteriocin

produced by *B. methylotrophicus* BM47 could be an effective mean for improving the shelf-life, quality, commercial appearance and safety of processed strawberries. These findings are important not only from a commercial point of view, but also represent a sustainable vision of the fruits postharvest, which is every day more requested. Nowadays, agricultural waste management is one of the major challenges, and both the edible coatings address this issue, prolonging the shelf-life of strawberries and at the same time reducing their wastes. Moreover, this preservation technique is simple, ecofriendly and can be applied without the need for expensive equipment and should therefore be considered for application in industrial fruit biopreservation.

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

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Table 1. Effects of carboxymethyl cellulose and carboxymethyl cellulose + bacteriocin edible coatings on the physicochemical parameters of fresh strawberries during storage at 4 °C and 75 % RH

Day	Coating	Parameters				
		ML/%	Decay/%	TSS/%	TA/%	pH
0	Control	n.d.	n.d.	8.4	0.9±0.03	3.63±0.02
	CMC	n.d.	n.d.	n.d.	n.d.	n.d.
	CMC+B	n.d.	n.d.	n.d.	n.d.	n.d.
4	Control	4.8	0	8.4	0.91±0.06	3.60±0.03
	CMC	3.7	0	8.4	0.92±0.05	3.61±0.04
	CMC+B	3.9	0	8.4	0.94±0.06	3.58±0.02
8	Control	9.11	10	8.7	0.96±0.07	3.54±0.01
	CMC	7.89	10	8.4	0.97±0.04	3.51±0.05
	CMC+B	7.83	0	8.4	0.99±0.05	3.41±0.03
12	Control	12.34	20	9.1	0.99±0.06	3.41±0.06
	CMC	10.46	10	8.6	1.01±0.08	3.40±0.05
	CMC+B	10.5	0	8.6	1.09±0.07	3.34±0.04
16	Control	17.37	60	9.4	1.10±0.04	3.32±0.07
	CMC	14.46	40	8.9	1.12±0.06	3.30±0.02
	CMC+B	14.41	20	8.9	1.13±0.08	3.24±0.01

CMC - carboxymethyl cellulose (0.5 %); B – bacteriocin of *B. methylotrophicus* BM47; ML – mass loss; TSS - total soluble solids; TA - titratable acidity; n.d. – not determined; ± - standard deviation (±SD).

Table 2. Effects of carboxymethyl cellulose and carboxymethyl cellulose + bacteriocin edible coatings on organic acid content of fresh strawberries during 16 days of storage at 4°C and 75 % RH

Day	Coating	Organic acid, mg/100 g fm		
		Ascorbic acid	Malic acid	Citric acid
0	Control	30.5±0.06	22.0±0.09	54.5±0.04
	CMC	n.d.	n.d.	n.d.
	CMC+B	n.d.	n.d.	n.d.
4	Control	30.0±0.08	21.5±0.07	56.0±0.05
	CMC	28.5±0.09	21.3±0.03	57.2±0.02
	CMC+B	28.2±0.01	20.6±0.04	58.5±0.09
8	Control	29.0±0.05	21.4±0.07	59.5±0.06
	CMC	27.9±0.08	21.0±0.02	60.4±0.04
	CMC+B	27.4±0.09	20.5±0.04	61.3±0.01
12	Control	25.7±0.01	19.6±0.05	61.5±0.08
	CMC	24.9±0.07	19.0±0.03	63.6±0.02
	CMC+B	24.5±0.04	18.8±0.09	65.1±0.06
16	Control	17.0±0.03	18.7±0.08	69.8±0.05
	CMC	16.0±0.06	18.2±0.01	71.7±0.04
	CMC+B	15.7±0.08	18.0±0.07	71.8±0.02

CMC - carboxymethyl cellulose (0.5 %); B – bacteriocin of *B. methylotrophicus* BM47; n.d. – not determined; ± - standard deviation (±SD).

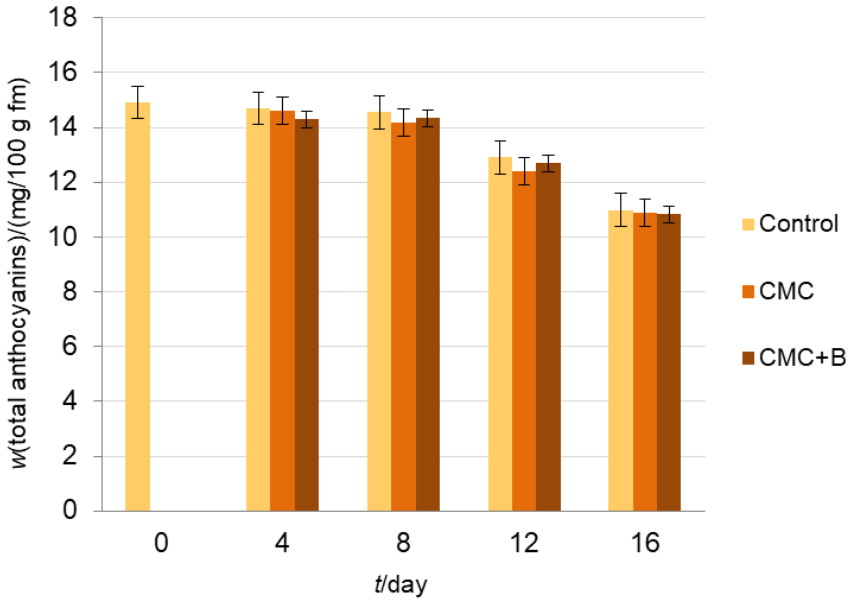

Figure 1. Effects of carboxymethyl cellulose (CMC) and carboxymethyl cellulose + bacteriocin (CMC+B) edible coatings on total anthocyanins content of fresh strawberries during 16 days of storage at 4°C and 75 % RH (error bars indicate standard deviation)

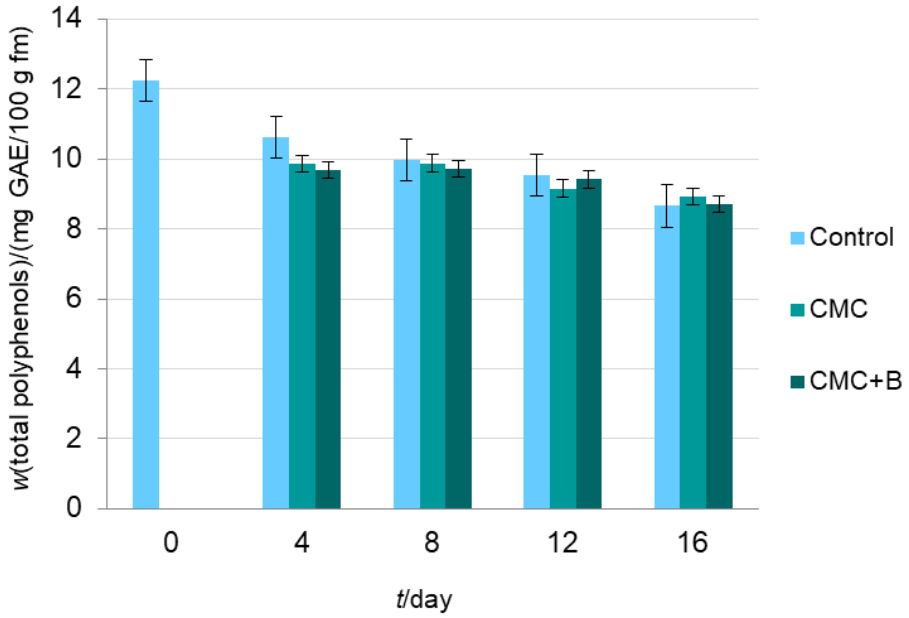

Figure 2. Effects of carboxymethyl cellulose (CMC) and carboxymethyl cellulose + bacteriocin (CMC+B) edible coatings on total phenolic content of fresh strawberries during 16 days of storage at 4°C and 75 % RH (error bars indicate standard deviation)

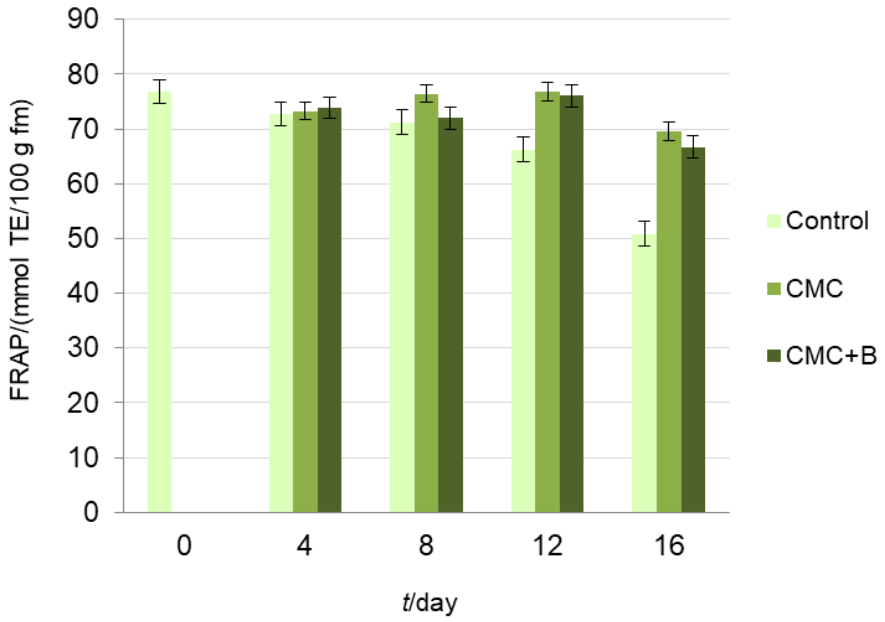

Figure 3. Effects of carboxymethyl cellulose (CMC) and carboxymethyl cellulose + bacteriocin (CMC+B) edible coatings on antioxidant activity of fresh strawberries during 16 days of storage at 4°C and 75 % RH (error bars indicate standard deviation)