Research Article

Received: 3 May 2017 Revised: 16 August 2017 Accepted article published: 24 October 2017 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.8751

Influence of polysaccharide-based edible coatings as carriers of prebiotic fibers on quality attributes of ready-to-eat fresh blueberries

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Abstract

BACKGROUND: Little information is available regarding the effect of dietary fibers added into edible coatings on quality attributes of ready-to-eat fruits. The aim of this study was to evaluate the effects of sodium alginate (AL) and chitosan (CH) edible coatings enriched with four different dietary fibers (apple fiber, orange fiber, inulin and oligofructose) on microbiological, nutritional, physico-chemical and sensorial properties of ready-to-eat fresh blueberries stored for 18 days at 5 °C.

RESULTS: The most encouraging results were found for CH coatings (with and without fibers) which significantly inhibited the growth of mesophilic bacteria and yeasts/molds (reductions up to 1.9 log CFU g^{-1}), reduced decay rate by more than 50%, enhanced antioxidant properties, retained fruit firmness, delayed off-odor development and improved overall visual quality of blueberries. Oligofructose and orange fiber added to CH coatings enhanced antioxidant properties of fruits and allowed higher reductions in yeast/mold counts compared to the use of CH alone. CH-based coatings enriched with inulin, oligofructose and apple fiber extended sensory shelf life of blueberries by 6 days. AL coatings (with and without fiber) allowed delaying fungal decay and also retaining antioxidant properties but did not improve the microbiological and sensory quality of fruits.

CONCLUSION: The results proved that fiber-enriched CH treatments allowed the maintenance of freshness and the improvement of the quality of ready-to-eat blueberries. It might be an interesting option to offer consumers a healthy product with prebiotic potential and an extended shelf life.

Keywords: fresh blueberries; prebiotic fiber; edible coatings; quality parameters; chitosan

INTRODUCTION

As a response to consumers' demand for healthy, fresh-like and easy-to-prepare products, in conjunction with consumers' lifestyle changes, a wide variety of minimally processed fruits and vegetables has been developed. The marketing of these types of foods continues to inrease mainly due to their freshness, economic handling and attractive presentation.[1] This trend parallels the increase in the demand for food products with health-promoting properties beyond the general provision of essential nutrients.[2] In particular, blueberries (*Vaccinium* spp.) are among the most popular berries in retail markets and are sold in fresh and processed forms for various food applications. Blueberries are rich in flavonoids and phenolic acids, which show relevant biological effects including antioxidant and anticarcinogenic properties and a protective effect against chronic diseases, especially cardiovascular diseases.[3] On the other hand, blueberries are very perishable after harvest; loss of firmness and microbial decay are two major detrimental factors during postharvest storage.[4]

Researchers and fruit processors are continuously looking for methods that contribute to minimizing the deleterious reactions triggered by mechanical damage, while keeping the fresh-like properties of the raw produce. Among these, edible coatings have a great potential to develop high-quality ready to eat fruits with an extended shelf life. Edible coatings (ECs) would provide a barrier to control moisture transfer, gas exchange and oxidation processes on the surface of fruits and vegetables.[5] ECs allow a delay in deterioration and also offer protection from physical damage of produce caused by mechanical impact. Hydrocolloids such as proteins and long-chain polysaccharides are the most suitable materials to produce coatings with appropriate structural properties.[6] ECs have been studied for preserving quality and extending shelf life of fresh berries including blueberries.[3,7–9] Chitosan, a linear polymer of 2-amino-2-deoxy-β-D-glucan, is a deacetylated form of chitin, a naturally occurring cationic biopolymer found mainly

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as the shell component of crustaceans. Chitosan is one of the most promising coating materials for fresh produce because of its excellent film-forming property, broad antimicrobial activity, and compatibility with other substances or functional ingredients. Moreover, alginates are the major structural polysaccharides of Phaeophyceae brown seaweed. They possess a good film-forming property, producing uniform, transparent, and water soluble films.[10]

The development of ECs as carriers of functional ingredients and additives, such as nutraceuticals, antimicrobial and antioxidant agents, colorants and flavors is considered a promising alternative for maintaining freshness and improving quality of fresh-cut fruits and vegetables.[10–12] Calcium and vitamin E were added to xanthan gum and applied on carrots.[13] Also, chitosan coatings enriched with high concentrations of minerals or vitamin E were formulated to fortify fruits and vegetables. Such coatings were applied on fresh and frozen strawberries and raspberries.[14,15]

Dietary fiber is an essential nutrient in our diet which has been related to risk reduction of a number of chronic diseases including diabetes, heart diseases, and certain cancers.[16] Fibers from fruit and greens are especially used as functional food additives due to their prebiotic properties, promoting the growth of healthy bacteria in the gut. Particularly, dietary fibers obtained from apple fruits have a higher quality than those extracted from cereal sources due to a higher solubility and greater antioxidant properties.[17] Orange fiber constitutes a material rich in pectin, with potential to reduce blood cholesterol levels and also to affect glucose metabolism. Moreover, this fiber can be used as a gelling agent.[18] Inulin and oligofructose, indigestible polysaccharides, are present in many vegetables, fruits and cereals and used in a wide range of food formulations due to both technological and nutritional benefits associated.[19,20]

Little information is available regarding the impact of dietary fibers incorporated into edible films and coatings on quality of ready-to-eat fruits. A recent study demonstrated that gellan gum ECs enriched with apple fiber enhanced antioxidant properties, microbiological and sensory quality of fresh-cut 'Golden Delicious' apples.[12] As far as we know, the incorporation of dietary fibers into ECs applied for preserving quality and improving nutritional properties of ready-to-eat fresh blueberries has not been studied yet. Therefore, the purpose of this work was to evaluate the effect of different polysaccharide-based ECs enriched with dietary fibers on microbiological, nutritional, physico-chemical and sensory properties of fresh blueberries.

EXPERIMENTAL

Materials

Fresh blueberries (*Vaccinium corymbosum* L.) cv. Emerald were purchased from Compañia Industrial Frutihortícola S.A., a local company which processes fruits and vegetables in Sierra de los Padres, Buenos Aires province (Argentina). The fresh fruits were of commercial maturity and were stored at 5 ± 1 °C for a few hours until processing. Food-grade sodium alginate (Keltone LV, ISP, San Diego, CA, USA) and medium molecular weight chitosan (deacetylation degree 98%; ACOFAR, Mar del Plata, Argentina) were the carbohydrate biopolymers used for coating formulations. Glycerol (Biopack, Buenos Aires, Argentina) was added to the coatings as a plasticizer. Four different dietary fibers were incorporated to coating solutions. Apple fiber extract and orange fiber extract were supplied by Indulleida S. L. (Lleida, Spain). Apple fiber had a purity of 55.90% (w/w) with soluble and insoluble dietetic fiber content of 13.10% and 42.80% (w/w), respectively. Orange fiber had a purity of 43.37% (w/w) with soluble and insoluble dietetic fiber content of 23.13% and 20.24% (w/w), respectively. Inulin and oligofructose marketed as prebiotic fibers were supplied by Saporiti S.A. (Buenos Aires, Argentina).

Preparation of coating solutions

Chitosan (CH) solutions (20 g kg^{-1}) were prepared by dispersing chitosan powder in acetic acid solution (10 mL kg^{-1}) with magnetic stirring at 23 °C.[21] To achieve complete chitosan dispersion the solution was stirred overnight at room temperature. Sodium alginate (AL) was dissolved in distilled water (20 g kg^{-1}) by gently stirring at 70 °C until the solution became clear.[22] Glycerol was added at a concentration of 15 g kg^{-1} for AL and CH solutions. Coating solutions were prepared with and without the addition of four different dietary fibers. Apple fiber (AF) obtained from apple pomace and orange fiber (OF) were used at concentrations of 7 g kg^{-1} following the fiber:polysaccharide ratio suggested by Grigelmo-Miguel and Martín-Belloso.[17] Inulin (IN) and oligofructose (OL) were used at 40 g kg^{-1}, selected according to Moreira *et al.*[12] These authors selected the IN and OL concentrations based on a good solubility of fibers in the film-forming solutions and following the requirements of The Code of Federal Regulations (Title 21, Part 101.54) for prebiotic effect of functional foods.

Fruit coating

Blueberries were selected based on their uniformity of size and color. Rotten and damaged fruits were eliminated. Blueberries were first washed using tap water for 30 s, drained and air-dried on a stainless steel screen for 30 min prior to coating application. Subsequently, fruits were dipped into the chilled (5 °C) polysaccharide solutions (CH and AL with and without the addition of IN, OL, AF and OF) for 2 min. The excess of coating material was allowed to drip off for 1 min. Only alginate-coated fruits were submerged again for 2 min in a calcium chloride (20 g kg^{-1}) cross-linking solution.[12] Coated blueberries were air dried at room temperature on a stainless steel screen for 30 min. Uncoated samples dipped into distilled water were used as a reference (control). Ninety grams of treated fruit (45 – 50 units) were placed into 300 mL polypropylene containers (Boulevares SRL, Córdoba, Argentina). Containers were covered with a plastic film (PD 960, Sealed air Cryovac; Buenos Aires, Argentina) with an O_2 permeability of 7000 mL m^{-2} day^{-1} and CO_2 permeability of 20 000 mL m^{-2} day^{-1}, sealed and stored in darkness at 5 ± 1 °C. Three containers (three independent experimental units) for each combination coating-fiber or coating without fiber were randomly removed from storage and used for experimental determinations at each sampling time (0, 6, 12 and 18 days of refrigerated storage).

Microbiological analysis and fruit decay

The growth of naturally occurring microbial populations on fresh blueberries was evaluated over refrigerated storage. A portion of 10 g of blueberries was aseptically removed from each container and transferred into sterile plastic bags. Samples were homogenized with 90 mL of sterile peptone water (1 g kg^{-1}) for 1 min in a stomacher blender. Serial dilutions (1:10) of each sample were made in peptone water (1 g kg^{-1}) and surface spread by duplicate. The enumeration of the microbial populations was performed by using the following culture media and culture conditions: mesophilics on plate count agar (PCA) incubated at 35 °C for

48 h; yeasts and molds on yeast–glucose–chloramphenicol (YGC) medium incubated at 25 °C for 5 days. All culture media were purchased from Britania, Buenos Aires, Argentina. Microbial counts were expressed as $\log_{10}$ CFU g^{-1} of fresh blueberries. Analyses were performed at each sampling time from three randomly sampled containers and at least two replicate counts were carried out for each container.

Fruit decay was visually evaluated immediately after removal from cold storage in each container at each sampling time. Berries with visible mold growth were considered decayed. Decay rate was expressed as percentage of fruit showing decay symptoms in each container.

Antioxidant assays

Ethanolic extracts were prepared from treated fruit samples to determine their total polyphenol content (TPC) and antioxidant capacity (AC) by DPPH assay. The extraction procedure was performed by homogenizing a sample of 10 g of treated blueberries with 20 mL of 80% ethanol. Then, the mixture was treated in a sonicator for 30 min and centrifuged at 10 000 × *g* for 15 min at 4 °C. The supernatant was collected and filtered using Whatman filter paper #1. The pellet was used to repeat the extraction procedure two more times. Extracts were prepared from fruit corresponding to three containers of each treatment at 0, 6, 12 and 18 days of storage.

Antioxidant properties of dietary fibers (AF, OF, IN and OL) were determined in order to evaluate the contribution of fibers to antioxidant status of coated blueberries. To carry this out, ethanolic extracts of fibers were prepared. An amount of 0.2 g of each fiber was homogenized with 10 mL of 80% ethanol, sonicated and centrifuged as mentioned above for blueberry extracts.

Total phenolic content

TPC was determined spectrophotometrically using the Folin–Ciocalteu reagent (FCR) according to the methodology proposed by Tomadoni *et al.*[23] Each sample extract properly diluted (200 μL) was added to 1000 μL of FCR (diluted 1:10). After 3 min of incubation at ambient temperature, 800 μL of 7.5% Na_2CO_3 solution was added and the reaction mixture was incubated for 2 h at the same temperature. The absorbance was measured at 765 nm using a UV–visible spectrophotometer (1601 PC UV–visible; Shimadzu Corporation, Kyoto, Japan) and TPC was calculated using gallic acid as standard. Assays were carried out triplicate. TPC of fruits was expressed as mg gallic acid equivalents (GAE) 100 g^{-1} of fresh blueberries. TPC of dietary fiber extracts was expressed as mg GAE g^{-1} of fiber extract.

DPPH radical scavenging activity

AC was studied by evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the method described by Tomadoni *et al.*[23] An ethanolic DPPH solution (100 μ mol L^{-1}) was used for determinations. Ethanol (0.1 mL) was mixed with 3.9 mL of DPPH solution to determine the initial absorbance of the DPPH solution (reference sample). Next, 0.1 mL of sample extract was added to 3.9 mL of DPPH solution. The mixture was shaken immediately and allowed to stand at ambient temperature in the dark for 60 min. Absorbance at 517 nm was measured. The % inhibition of DPPH was calculated according to the formula: % inhibition = $[(A_0 - A)/A_0] \times 100$, where A_0 and A are the absorbance values of the reference sample and the radical plus sample extract, respectively. Measurements were done in triplicate.

Surface color and firmness

Surface color was measured on blueberries using a colorimeter (RT Series; Lovibond, London, UK). Color was recorded using the CIE $L^*a^*b^*$ uniform color space, where L^* indicates lightness (whiteness or brightness/darkness), a^* indicates chromaticity on a green (−) to red (+) axis, and b^* indicates chromaticity on a blue (−) to yellow (+) axis (CIE, 1978). Color modification was evaluated through changes in lightness (L^*) and hue angle [hue° = arctan(b^*/a^*)] where 0° = red–purple, 90° = yellow, 180° = bluish green and 270° = blue. The colorimeter had been standardized against a white tile ($L^* = 97.63$, $a^* = 0.3133$, $b^* = 0.3192$). The measurements were made on 15 fruits corresponding to three containers per treatment and sampling date ($n = 15$). Fruit firmness was measured with a digital penetrometer (FHT-801; Test Equipment Depot, Melrose, MA, USA) using a 3.5 mm plunger diameter. Maximum strength required to penetrate the fruit on the calyx side was recorded and expressed as N cm^{-2}. The measurements were made on 15 fruits corresponding to three containers per treatment and sampling date ($n = 15$).

Sensory evaluation

Quantitative descriptive analysis was used to evaluate sensory attributes of blueberries at 0, 6, 12 and 18 days of storage. Sensory analysis was carried out as described by Alvarez *et al.*[24] with some modifications. Briefly, a panel comprised of nine members of the UNMdP Food Engineering Group aged 25–50 years and with experience in fruit and vegetable sensory quality carried out the evaluation of blueberries. In previous work sessions, preliminary sensory tests were performed to identify those defects most likely to appear due to prolonged storage of coated and uncoated blueberries. Panelists thoroughly discussed, defined each attribute to be evaluated and agreed on the use of the corresponding scales. The attributes evaluated were overall visual quality (OVQ), color, odor and odd-flavor. Blueberries were removed from storage conditions and tempered at room temperature before sensory evaluations. The coded (three-digit) samples were presented one at the time in random order to the members who sat at a round table and made independent evaluations. Evaluations were performed under artificial daylight-type illumination, at room temperature (22–24 °C). The intensity of the attributes evaluated was quantified on a 5 cm unstructured intensity scale. OVQ was scored from 0 (highly deteriorated aspect) to 5 (appealing/fresh aspect). Odor from 0 (intense off-odors) to 5 (fresh) and flavor from 0 (intense odd flavor) to 5 (typical/no odd flavor). The limit of acceptance was 2.5 (50% of the scale) indicating that a score below this limit was deemed to indicate end of shelf life.[24,25]

Statistical analysis

The experimental design used in this study was completely randomized with two factors, treatment (including each coating alone or in combination with dietary fibers) and storage time. Analysis of variance ANOVA ($P < 0.05$) was performed and differences between means were determined using the LSD (least significant difference) test with a 95% confidence level. Data were analyzed using InfoStat (v2013) statistical software (Universidad de Córdoba, Córdoba, Argentina).

RESULTS AND DISCUSSION

Functional coatings applied on blueberries

As a consequence of the coatings application to fresh blueberries, the weight gain averaged 50 g kg^{-1} using AL and 14 g kg^{-1} using CH. Thus, the use of AF and OF resulted in a fiber addition of approximately 370 mg kg^{-1} for alginate-coated blueberries and 90 mg kg^{-1} for chitosan-coated blueberries. Moreover, the use of IN and OL resulted in a fiber addition of approximately 1930 mg kg^{-1} for AL-coated blueberries and 670 mg kg^{-1} for CH-coated blueberries. This additional contribution of dietary fiber represents from 0.3% to 0.8% of the average recommended daily intake of fiber (25 g) by FAO/WHO,[26] based on a 100 g fiber-coated blueberries portion.

Microbial counts

Agents responsible for microbiological spoilage in fruits and derivatives can be bacteria, as well as yeasts and molds. The latter are considered the main spoilage agents due to the low pH of most fruits.[20] Mesophilic aerobic microorganisms (MES) are indicative of the endogenous microflora and the contamination undergone by the material; MES counts allow the estimation of total viable populations.[27] Figure 1 shows the changes in MES counts on fresh blueberries coated with CH or AL with and without added dietary fibers during refrigerated storage. The use of CH as coating on fresh blueberries, with or without added dietary fiber, had a significant effect ($P < 0.05$) on MES counts (Fig. 1a). Although the use of AL did not exert significant effects on this population ($P > 0.05$) and no differences were observed between treatments along the storage (Fig. 1b), CH-coated blueberries showed significantly lower initial MES counts (3.0 log CFU g^{-1}) compared to the uncoated control (4.7 log CFU g^{-1}) at day 0 (Fig. 1a). However, AL-coated blueberries exhibited MES counts ranging from 3.8 to 4.6 log CFU g^{-1}, which were similar to the counts initially found on uncoated blueberries (Fig. 1b). All CH-coated fruits, with and without added fiber, exhibited significantly lower MES counts compared to control (1.5–1.9 log reductions) on average throughout storage (Fig. 1c). The addition of fiber was generally not found to be relevant in terms of MES counts.

The changes in yeast and mold (YM) loads growing on fresh blueberries along refrigerated storage are shown in Fig. 2. As it was observed for MES, the use of CH, with or without added dietary fiber, significantly ($P < 0.05$) reduced YM growth on coated blueberries (Fig. 2a). However, when AL coatings were applied, no differences ($P > 0.05$) were observed between coated and uncoated samples along the storage (Fig. 2b). All CH-coated samples (with and without fibers) showed significantly lower YM counts compared to uncoated control (0.9–1.9 log reductions) on average throughout the storage. The addition of dietary fibers improved CH antimicrobial effect. The most significant inhibitory effects were observed in CH-OL and CH-OF coated samples, with reductions in the range of 0.8–1.0 log on average throughout the storage, compared to the use of CH alone. This result suggested that OF extract may contain bioactive compounds of plant origin with significant antifungal capacity that could act in conjunction with chitosan enhancing its inhibitory activity. Finally, for the entire storage period, CH- and AL-coated fruits with and without dietary fibers, as well as untreated control, showed microbial counts below the maximum limit of microorganisms (7 log CFU g^{-1}) allowed in minimally processed foods according to the Spanish Regulation.[28] Thus, after 18 days of cold storage, all samples continued to be safe for consumption and shelf life was not limited by microbial counts.

There is ample evidence that chitosan coating has the potential to prolong the storage life and control decay of fruits;[29] its antimicrobial capacity is mainly attributed to changes in cell permeability due to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membranes leading to the leakage of intracellular constituents.[29] In a recent study, Chiabrando and Giacolone[30] applied chitosan (20 g kg^{-1}) and alginate (15 g kg^{-1}) coatings alone and combined (15 and 10 g kg^{-1}, respectively) on fresh blueberries and recommended chitosan as the most effective treatment to reduce YM growth during cold storage.

Regarding the effect of inulin and apple fiber addition, our results are in agreement with those obtained by Moreira *et al*[12] who applied pectin, gellan gum and sodium alginate enriched with inulin and apple fiber as coatings on apple cubes and found that the addition of fiber extracts did not change the microbiological quality of coated apples.

Citrus fiber has better quality than other dietary fibers due to the presence of associated bioactive compounds, mainly polyphenols and flavonoids. Fernandez-Lopez *et al.*[31] described the phenolic composition of orange fiber obtained directly from by-products of an orange juice industry. The major phenolic compound found was hesperidin and others detected in minor amounts were eriocitrin, neoeriocitrin, rutin, luteolin-7-*O*-glucoside, diosmin, poncirin, hesperetin and neodiosmin. Moreover, Ortuño *et al.*[32] reported that hesperedin along with other flavonoids, such as naringin and nobiletin, isolated from citrus peel (orange and grapefruit) had a significant antifungal effect against *Penicillium digitatum*. These authors suggested that these compounds play an active role in the protection of fruits against pathogen attack. Regarding orange fiber as a food ingredient, it was applied on meat products and positive effects were found on quality with regard to reducing the microbial growth of unwanted microbes, therefore increasing the shelf-life of such products.[33,34] Particularly, Viuda-Martos *et al.*[34] applied orange fiber (10 g kg^{-1}) combined with oregano essential oil (0.2 g kg^{-1}) to preserve the quality of bologna sausages. This treatment significantly reduced lactic acid bacteria and aerobic bacteria growth. Similar results were found when OF was applied in combination with rosemary oil in mortadella.[33] Antimicrobial effects were attributed to flavonoids (mainly hesperidin) belonging to orange fiber and terpenes present in essential oils.

Decay rate

Decay rate of blueberries subjected to the different coating formulations was evaluated throughout the storage (Fig. 3). Those fruits with visible mold growth were considered decayed. All coatings applied (CH or AL with and without fibers) significantly reduced the decay rate of blueberries along the storage period compared to the control sample ($P < 0.05$). While control sample reached a decay rate of 23% at the first 6 days of storage, CH- and AL-coated samples showed 3% and 16%, respectively. On average, throughout the storage period, CH- and AL-based coatings reduced fungal decay by 54% and 30%, respectively, compared to the control. The inhibitory effect of CH was much more pronounced compared to AL and was consistent with the reductions observed on YM counts. Moreover, prebiotic fiber addition into the coating formulations did not produce any difference on the decay rate of samples compared to the use of CH and AL coatings without fiber (Fig. 3a and b). As concluded by several authors, coatings create a modified atmosphere on fruit surface that may inhibit microbial growth during postharvest storage,[35] resulting in a lower decay rate of coated fruits. Duan *et al.*,[7] working with blueberries cv.

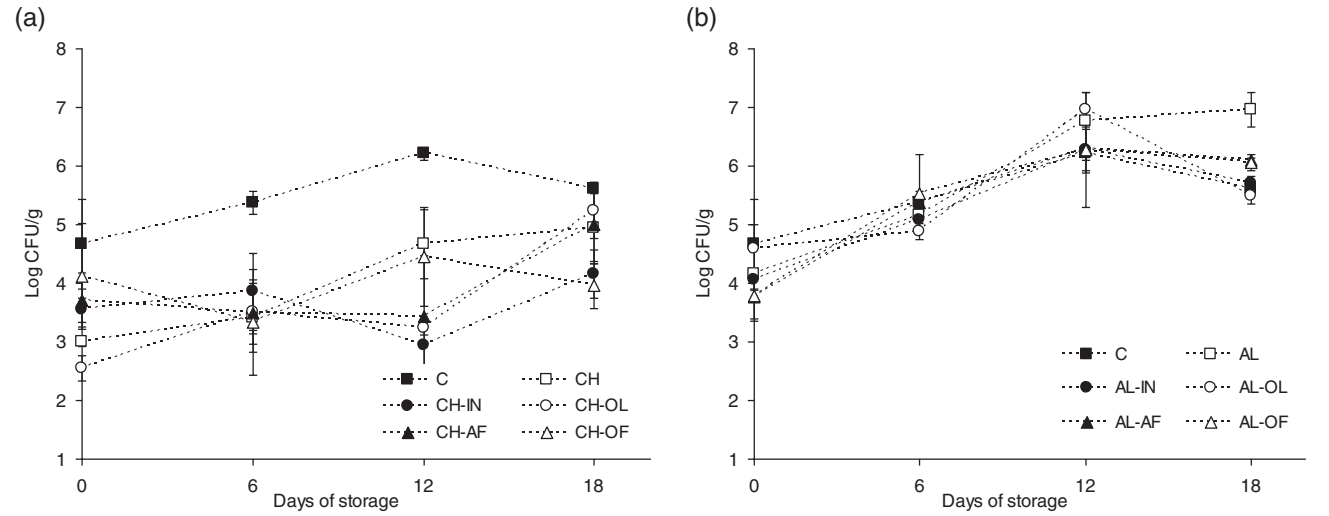

Figure 1. Effect of chitosan (a) and sodium alginate-based coatings (b) enriched with dietary fibers on mesophilic counts of fresh blueberries during 18 days of storage at 5 °C. Uncoated control (C), chitosan (CH), CH plus inulin (CH-IN), CH plus oligofructose (CH-OL), CH plus apple fiber (CH-AF), CH plus orange fiber (CH-OF). Alginate (AL), AL plus IN (AL-IN), AL plus OL (AL-OL), AL plus AF (AL-AF), AL plus OF (AL-OF). Data shown are the means ± standard deviation.

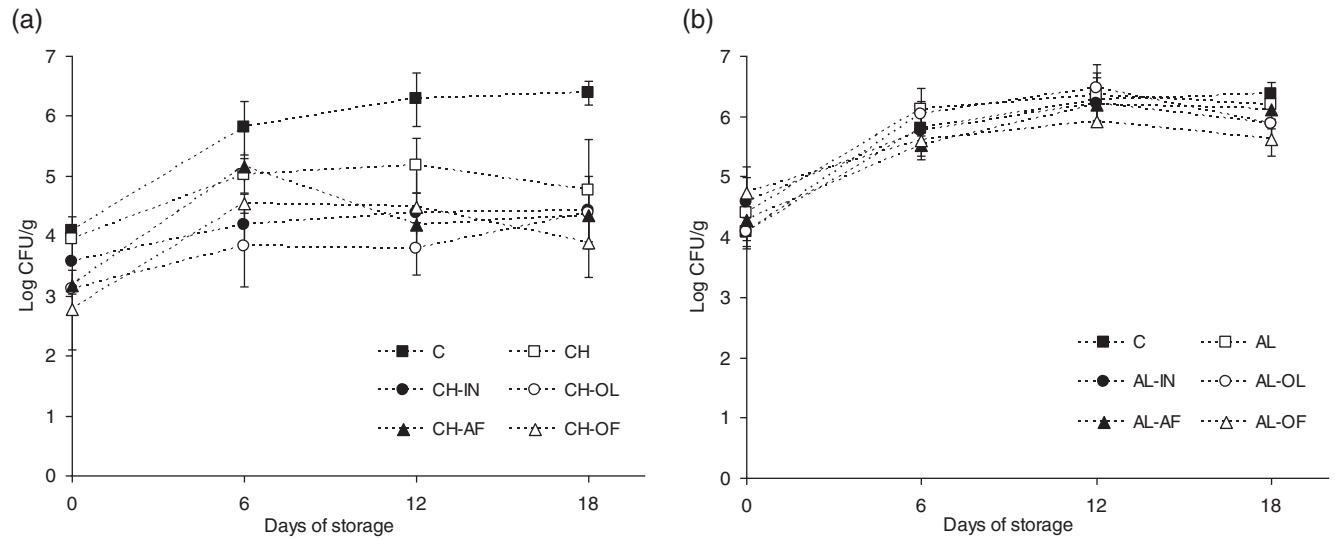

Figure 2. Effect of chitosan (a) and sodium alginate-based coatings (b) enriched with dietary fibers on yeast and mold counts of fresh blueberries during 18 days of storage at 5 °C. Uncoated control (C), chitosan (CH), CH plus inulin (CH-IN), CH plus oligofructose (CH-OL), CH plus apple fiber (CH-AF), CH plus orange fiber (CH-OF). Alginate (AL), AL plus IN (AL-IN), AL plus OL (AL-OL), AL plus AF (AL-AF), AL plus OF (AL-OF). Data shown are the means ± standard deviation.

Duke demonstrated that chitosan coating significantly lowered decay rate when compared to washed control fruits at days 9 and 12 of room temperature storage (fruits previously stored 1 week at 2 °C). The mechanisms by which chitosan coatings reduced the decay in whole (intact) strawberries appeared to be related to their fungistatic property (cellular damage to the molds and interference in the secretion of polygalacturonases) rather than to their ability to induce plant defense enzymes.[36] The stimulation of defense enzymes, such as chitinase, chitosanase, and β-1,3-glucanase was observed by these authors in fresh-cut strawberries, with a greater interaction between the coating material and the tissue compared to intact fruits.

Total phenolic content and antioxidant capacity

Blueberries contain a wide variety of phytochemicals, such as polyphenols and flavonoids, which have been suggested to provide important health benefits due to their antioxidant properties.[30] These compounds are not completely stable and, after harvest, they undergo changes during processing and storage, which may alter their biological activity.[37]

Figure 4 displays the changes in TPC and AC of CH- and AL-coated blueberries. A significant correlation effect was observed between TPC and AC of treated blueberries with a Pearson coefficient of 0.78 for CH-coated fruits and 0.70 for AL-coated fruits. The evolution of TPC and AC during storage in samples treated with different combinations of coatings and fibers did not follow a common pattern and was different for each treatment. All samples exhibited fluctuations in TPC and AC throughout storage (Fig. 4a–d). Piljac-Zegarac *et al.*[38] also observed some fluctuations in the antioxidant capacity of dark fruit juices during refrigerated storage. Particularly, CH samples (without fiber) showed higher TPC values compared to uncoated

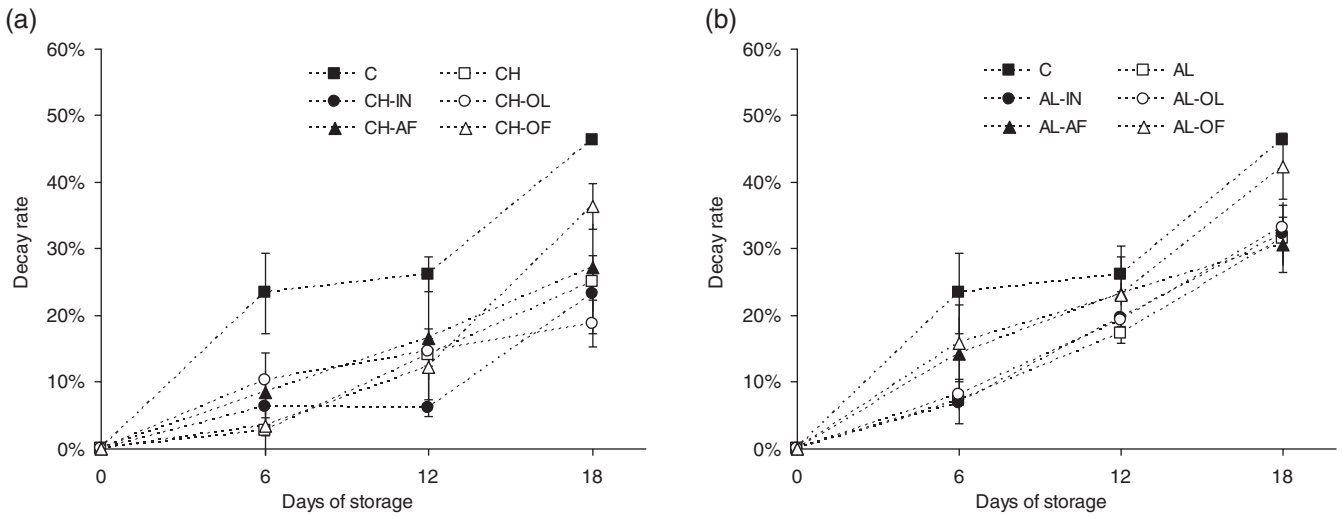

Figure 3. Decay rate of chitosan (a) and sodium alginate (b) coated blueberries enriched with dietary fibers during 18 days of storage at 5 °C. Uncoated control (C), chitosan (CH), CH plus inulin (CH-IN), CH plus oligofructose (CH-OL), CH plus apple fiber (CH-AF), CH plus orange fiber (CH-OF). Alginate (AL), AL plus IN (AL-IN), AL plus OL (AL-OL), AL plus AF (AL-AF), AL plus OF (AL-OF). Data shown are the means ± standard deviation.

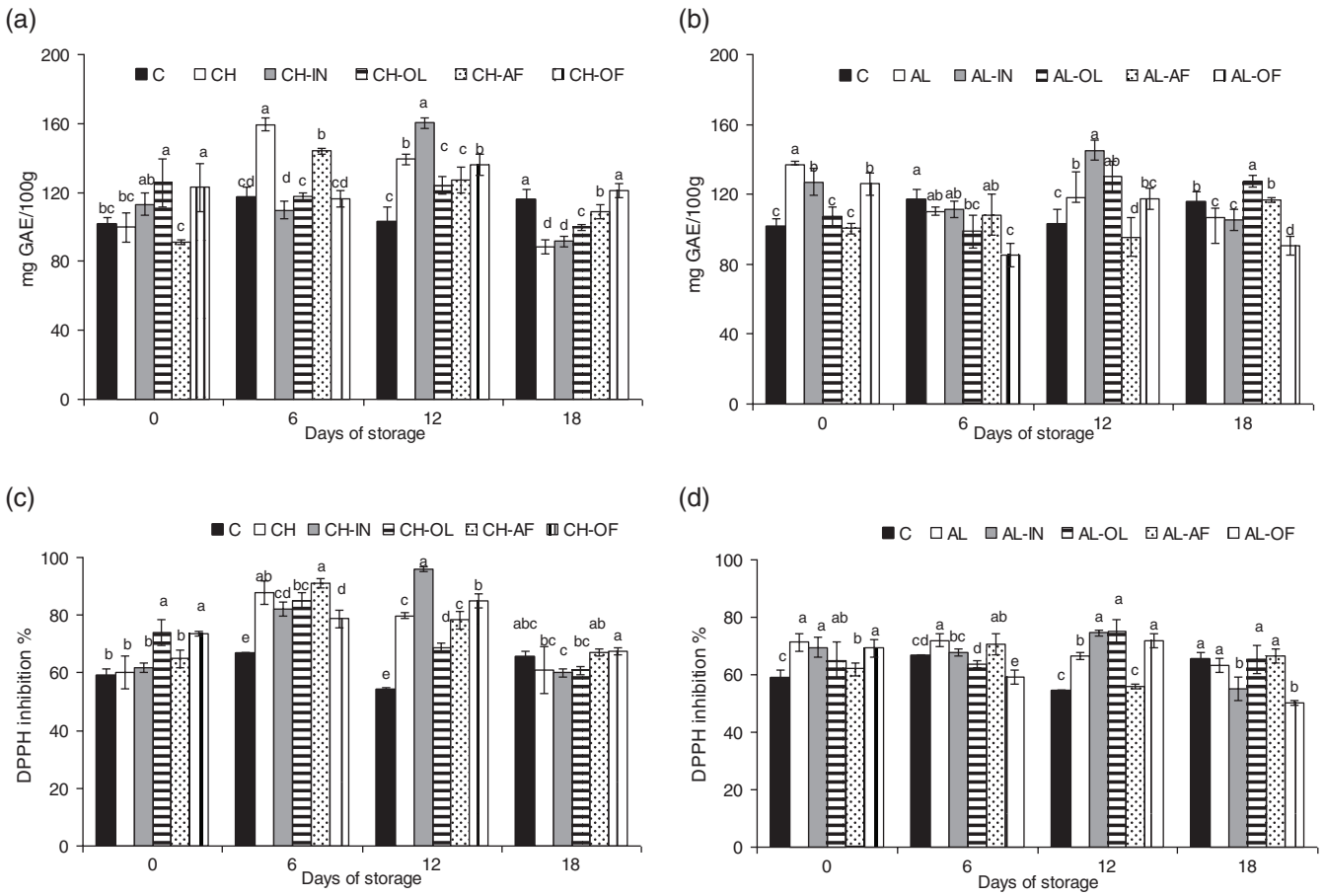

Figure 4. Total polyphenol content of chitosan (a) and sodium alginate (b) coated blueberries and antioxidant capacity (by DPPH assay) of chitosan (c) and sodium alginate (d) coated blueberries enriched with dietary fibers during 18 days of storage at 5 °C. Uncoated control (C), chitosan (CH), CH plus inulin (CH-IN), CH plus oligofructose (CH-OL), CH plus apple fiber (CH-AF), CH plus orange fiber (CH-OF). Alginate (AL), AL plus IN (AL-IN), AL plus OL (AL-OL), AL plus AF (AL-AF), AL plus OF (AL-OF). Data shown are the means ± standard deviation. Different letters indicate significant differences ($P < 0.05$) among treatments at each sampling time.

Table 1. Color parameters *L** and hue of fresh blueberries stored for 18 days at 5 °C as affected by the application of chitosan and alginate-based edible coatings enriched with dietary fibers

Parameter	Treatment	Storage time (days) 0	6	12	18	Mean
*L**	C	25.67 ± 1.30	26.64 ± 1.56	27.35 ± 1.83	27.97 ± 1.92	26.80[a]
	CH	25.27 ± 2.03	25.14 ± 2.03	25.88 ± 2.59	26.73 ± 1.58	25.76[e]
	CH-IN	26.01 ± 1.46	26.05 ± 1.59	27.43 ± 2.96	26.95 ± 2.33	26.61[ab]
	CH-OL	26.06 ± 1.75	25.76 ± 1.89	24.99 ± 1.89	26.65 ± 1.36	25.79[be]
	CH-AF	26.54 ± 2.02	24.99 ± 1.85	25.04 ± 1.68	27.12 ± 2.00	25.92[be]
	CH-OF	26.06 ± 1.76	24.88 ± 1.78	24.85 ± 2.17	26.59 ± 1.50	25.60[e]
Hue (°)	C	255.68 ± 3.99	259.42 ± 7.55	254.47 ± 6.13	253.33 ± 8.70	NS
	CH	254.30 ± 5.03	261.11 ± 8.33	251.76 ± 7.31	264.09 ± 15.18	
	CH-IN	257.03 ± 9.44	253.94 ± 6.01	253.05 ± 5.68	255.59 ± 6.01	
	CH-OL	258.80 ± 11.22	255.43 ± 7.08	256.42 ± 5.68	258.74 ± 9.58	
	CH-AF	259.23 ± 10.37	256.32 ± 7.27	253.79 ± 4.57	254.99 ± 4.63	
	CH-OF	258.08 ± 5.62	257.16 ± 9.66	254.15 ± 10.3	258.79 ± 10.14	
*L**	C	25.67 ± 1.30	26.64 ± 1.56	27.35 ± 1.83	27.97 ± 1.92	NS
	AL	27.98 ± 2.13	26.24 ± 1.99	27.60 ± 1.83	27.62 ± 1.65	
	AL-IN	27.14 ± 1.00	25.33 ± 2.46	27.01 ± 2.06	29.55 ± 2.65	
	AL-OL	26.66 ± 1.46	26.96 ± 1.71	27.35 ± 1.68	29.59 ± 1.87	
	AL-AF	26.35 ± 1.89	26.02 ± 1.11	27.30 ± 2.85	29.45 ± 1.48	
	AL-OF	27.74 ± 1.45	26.73 ± 1.33	26.93 ± 1.06	27.90 ± 1.48	
Hue (°)	C	255.68 ± 3.99	259.68 ± 2.04	254.47 ± 6.13	253.33 ± 8.70	NS
	AL	254.32 ± 5.86	255.42 ± 3.99	255.40 ± 3.19	263.06 ± 13.27	
	AL-IN	255.02 ± 2.29	258.85 ± 7.47	254.58 ± 5.44	257.01 ± 7.79	
	AL-OL	253.49 ± 3.61	252.73 ± 3.03	254.42 ± 5.75	256.24 ± 2.98	
	AL-AF	255.28 ± 3.15	254.89 ± 6.68	259.45 ± 4.28	260.38 ± 10.24	
	AL-OF	252.37 ± 2.66	256.08 ± 5.71	252.04 ± 2.98	255.60 ± 5.36	

Data is shown as means ± standard deviations.
Mean values with different letters indicate significant differences ($P < 0.05$) between treatments.
NS, treatment was a non-significant factor ($P > 0.05$).

control (C) for the first 12 days. Moreover, CH-OL and CH-OF treatments significantly increased ($P < 0.05$) initial TPC compared to C and CH (day 0). Only the effect of CH-OF was generally maintained during storage (Fig. 4a). In AL-coated samples, fiber addition did not increase TPC of blueberries compared to AL sample (without fiber) ($P > 0.05$). However, AL sample showed higher TPC values at days 0 and 12 compared to C values (Fig. 4b).

Regarding antioxidant activity of samples studied by DPPH scavenging assay, CH coatings with and without fibers significantly increased AC of fruits compared to C samples for the first 12 days of storage. In accordance with the results observed for TPC, CH-OL and CH-OF showed the highest initial AC values. Also, the addition of IN, AF and OF to CH coatings enhanced AC of CH-coated fruits on average throughout storage (comparison of treatments regardless of the time of storage). With regard to AL samples, alginate coating allowed the maintenance of slightly higher AC compared to C for the first 12 days of storage. However, the addition of IN, OL and OF to alginate coatings had a significant effect only at day 12 showing higher AC values compared to AL samples.

Regarding CH and AL effects, our results are in agreement with Chiabrando and Giacolone.[30] These authors reported that chitosan and sodium alginate coatings retarded the decrease of phenolic content and antioxidant activity during cold storage period in fresh blueberries cv. O'Neal and cv. Berkeley. The same trend was observed by Wang and Gao[8] in strawberries using chitosan.

Dietary fiber extracts, mainly those obtained from fruit by-products may be an important source of chemical compounds with antioxidant power. Corresponding assays to determine TPC and AC (DPPH method) of all the dietary fibers used for coating formulations were performed. As a result, a considerable amount of TPC was observed for those fibers obtained from fruit sources: 3.5 ± 0.2 and 4.9 ± 0.2 mg GAE g^{-1} of fiber for AF and OF, respectively. Moreover, AF and OF containing phenolic compounds and other antioxidants possibly present, such as ascorbic acid, exerted a significant DPPH scavenging capacity; AF and OF showed inhibition percentages of 52.3 ± 1.4 and 31.5 ± 0.4, respectively. On the other hand, inulin is extracted from chicory roots, purified and dried. Oligofructose is also derived from chicory as inulin, with the addition of a hydrolysis step after extraction.[39] As expected, IN and OL showed non-significant amounts of phenolic compounds. Also, a non-significant activity against DPPH radical was observed. Finally, the contribution of phenols and antioxidant capacity of AF and OF may partially explain the enhanced antioxidant properties of fiber enriched samples compared to those without fibers (Fig. 4). Also, chemical interactions between polysaccharides and fibers may occur and affect antioxidant status of treated fruits. This fact will be the object of future studies.

García *et al.*[40] studied the phytochemicals present in apple pomace and confirmed that this by-product is a valuable source of antioxidants with significant antioxidant capacity (DPPH and FRAP assays). The phytochemicals found by these authors were phenolic acids, such as chlorogenic, protocatechuic and caffeic acid and polyphenols, such as flavanols, dihydrochalcones (phloridzin and phloretin-20-xyloglucoside) and flavonols.[40] As detailed

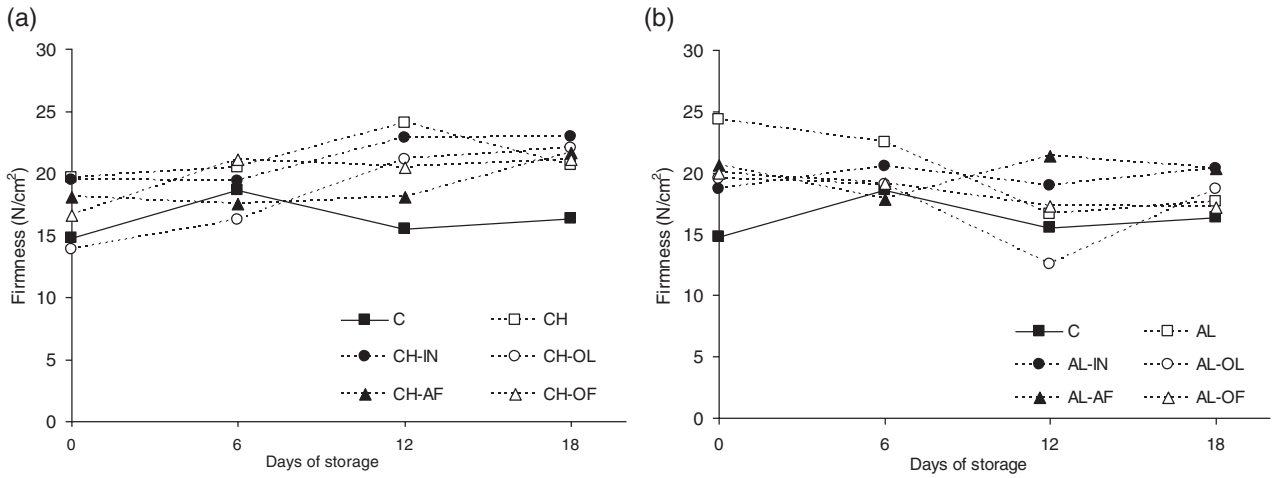

Figure 5. Effect of chitosan (a) and sodium alginate-based coatings (b) enriched with dietary fibers on firmness of blueberries during 18 days of storage at 5 °C. Uncoated control (C), Chitosan (CH), CH plus inulin (CH-IN), CH plus oligofructose (CH-OL), CH plus apple fiber (CH-AF), CH plus orange fiber (CH-OF). Alginate (AL), AL plus IN (AL-IN), AL plus OL (AL-OL), AL plus AF (AL-AF), AL plus OF (AL-OF).

above, orange fiber has also been found to contain many phytochemicals, with hesperidin the major phenolic compound and its antioxidant properties being demonstrated.[31,41]

Fiber extracts of different sources have been incorporated in foods from plant and animal origin in order to improve their functionality. Cassani *et al.*[20] found that the addition of oligofructose, inulin and apple fiber to strawberry juice (15 g kg^{-1}) significantly increased its antioxidant capacity when compared to untreated juice during cold storage. These authors concluded that the addition of fibers could increase the nutritional value of this product. Also, Moreira *et al.*[12] demonstrated that apple cubes coated with sodium alginate, pectin and gellan gum enriched with apple fiber (7 g kg^{-1}) maintained their antioxidant capacity in a better way during the first week of refrigerated storage. Furthermore, orange fiber added to meat products (bologna sausages and mortadella) showed effectiveness as an inhibitor of lipid oxidation, thereby improving oxidative stability and prolonging shelf-life of these foods.[33,34,42] Such activity was attributed to fiber phenolic compounds, hesperidin and narirutin, identified in treated meat products.[33]

On the other hand, changes in TPC of blueberries may have also been an endogenous cause due to the metabolic activity of fruits. Several authors related the increase in the antioxidant potential of fresh fruits to the accumulation of phenolic compounds caused by the induction of the phenylpropanoid metabolism.[12,43] This metabolism has been shown to be modulated by certain processing and storage conditions.[1] Thus, an uneven accumulation of phenolics during storage may explain differences between samples coated with different polysaccharide-based matrices. Also, the presence of antioxidants bonded to the fiber extracts, such as orange or apple fibers, could have a protective effect against oxidation and, at the same time, contribute to the activation of the production of the phenolic compounds by the fruit tissues.[17,44]

Color and firmness

Color parameters of blueberries as affected by chitosan and alginate-fiber enriched coatings are presented in Table 1. All CH coatings with and without fibers, with the exception of CH-IN, significantly reduced L^* of blueberries on average during storage ($P < 0.05$) compared to uncoated control. This luminosity decrease could be explained by the changes in the surface reflection properties that occurred when the blueberries were coated. The addition of OL, AF and OF to CH coatings had no significant effects on L^* when compared to CH treatment without fiber. Hue angle was not affected by CH treatments ($P > 0.05$). Regarding AL treatments (with and without fibers), fruit surface color did not change significantly ($P > 0.05$) during storage among different samples. Our results are in agreement with those of Chiabrando *et al.*,[30] who reported a significant reduction in luminosity of chitosan-coated blueberries compared to control along 45 days of storage at 0 °C, while an alginate coating did not exert significant changes. On the contrary, Yang *et al.*[3] reported that color parameters of blueberries were not significantly affected by the application of chitosan coatings enriched with different concentrations of a blueberry leaf extract.

Firmness is one of the most critical quality parameters influencing consumer acceptability and marketing of fresh fruit. Blueberries normally soften during the postharvest chain. Thus, shelf life decreases and fruit market value is reduced.[4] Figure 5 shows the firmness of blueberries treated with CH and AL coatings with and without fiber enrichment. Firmness evolution along the storage time is similar for the different samples. However, treatment factor significantly affected fruit firmness for CH-coated samples ($P < 0.05$) and did not exert effects when AL was used for coating formulation ($P > 0.05$). All CH-coated blueberries, with and without fibers, showed firmness values significantly higher than uncoated control ($P < 0.05$), on average, during storage period. Firmness retention due to CH treatments was more marked from day 12 until the end of the storage period (Fig. 5a). In addition, the inhibitory effect of CH on the growth of YM and the reduction on fruit decay rate previously described may contribute to firmness maintenance of blueberries. Previous studies reported that chitosan coatings were able to delay loss of firmness during postharvest cold storage for blueberries.[4,7] Finally, prebiotic fiber addition into the coating formulations did not produce any difference on firmness compared to the use of CH and AL coatings without fibers.

Sensory evaluation and shelf life

Combining edible coatings with nutraceutical ingredients as dietary fibers is a good strategy for the development of functional fruit-based foods. However, sensory appeal of odor, flavor and appearance of the treated fruit might be affected positively

Table 2. Sensory attributes of fresh blueberries stored for 18 days at 5 °C as affected by the application of chitosan and alginate-based edible coatings enriched with dietary fibers

Parameter	Treatment	Storage time (days) 0	6	12	18	
OVQ	C	4.4 ± 0.3^{a}	3.2 ± 0.2^{a}	3.0 ± 0.7^{a}	0.8 ± 0.6^{d}	
	CH	4.6 ± 0.3^{a}	3.7 ± 0.8^{a}	2.6 ± 0.2^{a}	2.4 ± 0.3^{b}	
	CH-IN	4.1 ± 0.6^{a}	3.5 ± 0.8^{a}	2.8 ± 0.6^{a}	3.0 ± 0.4^{a}	
	CH-OL	4.1 ± 0.6^{a}	3.0 ± 0.4^{a}	2.6 ± 0.3^{a}	2.5 ± 0.0^{b}	
	CH-AF	4.7 ± 0.3^{a}	3.6 ± 0.7^{a}	3.3 ± 0.8^{a}	2.6 ± 0.2^{ab}	
	CH-OF	4.7 ± 0.3^{a}	3.3 ± 0.6^{a}	2.8 ± 0.6^{a}	1.9 ± 0.4^{e}	
Odor	C	4.6 ± 0.2^{ab}	2.2 ± 0.4^{e}	2.1 ± 0.5^{a}	0.8 ± 0.1^{d}	
	CH	4.6 ± 0.5^{a}	3.5 ± 0.6^{b}	2.5 ± 0.6^{a}	2.8 ± 0.3^{a}	
	CH-1 N	4.5 ± 0.6^{ab}	4.4 ± 0.1^{a}	2.8 ± 0.2^{a}	2.6 ± 0.3^{ab}	
	CH-OL	3.9 ± 0.6^{b}	3.6 ± 0.6^{ab}	203 ± 0.6^{a}	1.8 ± 0.2^{e}	
	CH-AF	4.2 ± 0.6^{ab}	4.4 ± 0.2^{ab}	2.2 ± 0.5^{a}	2.4 ± 0.3^{b}	
	CH-OF	4.6 ± 0.4^{a}	4.3 ± 0.4^{ab}	2.1 ± 0.4^{a}	2.3 ± 0.2^{be}	
Flavor	C	4.5 ± 0.2	NS			
	CH	4.5 ± 0.5				
	CH-IN	4.2 ± 0.6				
	CH-OL	4.0 ± 0.6				
	CH-AF	4.2 ± 0.5				
	CH-OF	4.4 ± 0.4				
OVQ	C	4.4 ± 0.3	3.2 ± 0.2	3.0 ± 0.7	0.8 ± 0.8	NS
	AL	4.2 ± 0.4	3.1 ± 0.4	3.0 ± 0.6	1.0 ± 0.1	
	AL-IN	4.3 ± 0.4	3.0 ± 0.4	2.3 ± 0.6	0.9 ± 0.2	
	AL-OL	4.4 ± 0.2	2.3 ± 0.7	2.5 ± 0.7	0.8 ± 0.3	
	AL-AF	4.3 ± 0.4	3.1 ± 0.4	1.6 ± 0.7	1.6 ± 0.1	
	AL-OF	4.3 ± 0.4	2.7 ± 0.4	3.0 ± 0.8	1.7 ± 0.1	
Odor	C	4.6 ± 0.2	2.2 ± 0.4	2.1 ± 0.5	0.8 ± 0.1	NS
	AL	4.4 ± 0.3	1.9 ± 0.8	2.7 ± 0.7	1.0 ± 0.2	
	AL-IN	4.2 ± 0.7	1.6 ± 0.8	2.3 ± 0.7	1.1 ± 0.2	
	AL-OL	4.2 ± 0.3	1.3 ± 0.7	1.1 ± 0.6	1.1 ± 0.1	
	AL-AF	4.1 ± 0.7	1.9 ± 0.7	1.6 ± 0.6	1.8 ± 0.3	
	AL-OF	3.9 ± 0.8	1.8 ± 0.3	2.5 ± 0.4	1.9 ± 0.3	
Flavor	C	4.5 ± 0.2	NS			
	AL	4.5 ± 0.3				
	AL-IN	4.2 ± 0.4				
	AL-OL	4.03 ± 0.3				
	AL-AF	4.1 ± 0.5				
	AL-OF	4.0 ± 0.6				

Data is shown as means ± standard deviations.
Mean values with different letters indicate significant differences ($P < 0.05$) at each storage time.
NS, treatment was a non-significant factor ($P > 0.05$).

or negatively. The impact of exogenous flavor caused by the coating materials, the unattractive surface appearance of coatings and other factors may affect consumer acceptance of the coated products.[10] Thus, the impact of edible coatings and natural ingredients on sensory quality parameters and acceptability of minimally processed fruits should be studied. Flavor evaluations were made on blueberry samples after treatments application (day 0). As a result, no significant differences ($P > 0.05$) were observed between fiber enriched coatings and controls (uncoated and coated without fiber) (Table 2); all samples showed flavor scores near to the optimum value (5) indicating that strange flavors due to added fibers and coatings were not detected by panelists.

According to Aked,[45] appearance is the most important quality attribute of fresh and minimally processed produce, with primary concern for size and color uniformity, glossiness and absence of defects in shape or skin. Some aspects influencing appearance are wilting, loss of surface gloss, skin wrinkling, and skin blemishes caused by natural senescence or the growth of microorganisms.[10] Overall visual quality (OVQ) and odor evaluations were made on blueberries along the storage period. Results are shown in Table 2. Both OVQ and odor scores of all samples decreased significantly as storage time increased, indicating a gradual deterioration of sensory quality. All CH-based coatings delayed the development of off-odors throughout the whole storage period. Also, these treatments significantly improved OVQ of fruits when compared to uncoated samples at the end of the storage. At day 18, only those samples coated with CH enriched with IN, OL and AF showed OVQ scores above the acceptability level and, consequently, an extended sensory shelf life, while the remaining samples were unacceptable. Therefore, improvements in sensory

parameters observed for CH-coated fruits (regardless of the addition of fibers) could be closely related to the inhibitory effect of CH on YM growth, the reduction on fruit decay rate and the firmness retention previously described in this work. Finally, AL treatments (with and without fibers) did not exert significant effects on sensory attributes of blueberries ($P > 0.05$) during storage.

CONCLUSION

The impact of chitosan and sodium alginate edible coatings enriched with four different dietary fibers on microbiological, nutritional, physico-chemical and sensorial properties of ready-to-eat blueberries was studied. CH coatings (regardless of fiber addition) greatly controlled mesophilic bacteria and yeasts/molds growth, reduced decay, enhanced antioxidant properties, retained fruit firmness, delayed off-odor development and improved overall visual quality of blueberries. OL and OF added to CH had a positive effect on the nutritional and microbiological quality of treated fruits. Moreover, CH-based coatings enriched with IN, OL and AF extended the sensory shelf life by 6 days. AL coatings (with and without fiber) allowed fungal decay to be delayed and antioxidant properties to be retained but did not improve the microbiological and sensory quality of fruits. Finally, the use of functional edible coatings based on CH allowed the maintenance of freshness and the improvement of the quality of ready-to-eat fruits, which might be an interesting option to offer consumers a healthy product with prebiotic potential and an extended shelf life.

ACKNOWLEDGEMENTS

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and Universidad Nacional de Mar del Plata (UNMdP).

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