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Effect of chitosan–*Aloe vera* coating on postharvest quality of blueberry (*Vaccinium corymbosum*) fruit

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ABSTRACT

The present study was carried out to evaluate the effect of chitosan-based edible coatings with *Aloe vera* extract on the postharvest blueberry fruit quality during storage at 5 °C. Firstly, *A. vera* fractions (pulp and liquid) were extracted from leaves and evaluated in terms of antifungal and antioxidant capacities. The choice of the most adequate chitosan and *A. vera* fraction concentrations to be incorporated in coating formulation was made based on the wettability of the corresponding coating solutions. Coatings with 0.5% (w/v) chitosan + 0.5% (w/v) glycerol + 0.1% (w/v) Tween 80 + 0.5% (v/v) *A. vera* liquid fraction presented the best characteristics to uniformly coat blueberry surface. Physico-chemical (i.e., titratable acidity, pH, weight loss) and microbiological analyses of coated blueberries (non-inoculated or artificially inoculated with *Botrytis cinerea*) were performed during 25 d. Microbiological growth and water loss levels were approximately reduced by 50% and 42%, respectively, in coated blueberries after 25 d compared to uncoated blueberries. After 15 d, weight loss values were 6.2% and 3.7% for uncoated and chitosan–*A. vera* coated blueberries, respectively. Uncoated fruits presented mold contamination after 2 d of storage (2.0 ± 0.32 log CFU g^{-1}), whilst fruits with chitosan-based coatings with *A. vera* presented mold contamination only after 9 d of storage (1.3 ± 0.35 log CFU g^{-1}). Overall, coatings developed in this study extend blueberries' shelf-life for about 5 d, demonstrating for the first time that the combination of chitosan and *A. vera* liquid fraction as edible coating materials has great potential in expanding the shelf-life of fruits.

1. Introduction

Blueberries (*Vaccinium corymbosum*) are currently one of the most valuable fruits worldwide due to its organoleptic and nutritional properties. However, from the moment that blueberries are harvested they are very susceptible to structural, nutritional and biochemical changes. These postharvest changes can be accelerated principally, by water loss and action of microorganisms, mostly by fungal outbreaks (e.g., *Botrytis cinerea*) (Yang et al., 2014).

In recent years, edible films and coatings have been considered one technology with great potential to improve safety of food and to protect it from the influence of external environmental factors, thus increasing its shelf life (Carneiro-da-Cunha et al., 2009; Cerqueira et al., 2011). This type of coatings can be a biodegradable alternative to the use of plastic packages, since they can create a protective barrier, semi-permeable to gases and water vapor, and also could reduce microbiological proliferation (Dutta et al., 2009). One of the main food application of edible coatings is on fruit surface, such as strawberry (Del-Valle et al., 2005), grapes (Valverde et al., 2005; Castillo et al., 2010), tropical fruits (Cerqueira et al., 2009), among others. The purpose is to create a more efficient system for fruit storage, aiming to reduce the degradation of qualitative aspects in the postharvest period and lower loss rates to extend shelf-life (Pinheiro et al., 2010). Also, the properties of the coatings can be enhanced using functional ingredients incorporated within such as antibrowning and antimicrobial agents, nutraceuticals, volatile precursors, and colors (Olivas and Barbosa-Cánovas, 2005). Other ingredients, such as

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preservatives, antioxidants, and firming agents can be added to coatings to improve microbial stability, appearance, and texture of coated product (Cerqueira et al., 2009; Bai and Plotto, 2012).

In order to improve the efficiency and stability of edible coatings/films it is essential to find adequate materials. Coatings/films can be produced using a wide variety of products, such as polysaccharides, proteins, lipids or resins, alone or, more often, in combination (Flores-López et al., 2015). Chitosan (1,4-linked 2-amino-2-deoxy-β-D-glucan) is one of the most widely used natural compounds in the edible coating production. Due to its characteristics such as high antimicrobial activity, biocompatibility, biodegradability and non-toxic profile, this polysaccharide has been studied for application in different areas, with primary emphasis on food and pharmaceutical industries; but also in medicine, agriculture, and environment (Pinheiro et al., 2010; Ruiz-Navajas et al., 2013; Jiang et al., 2014). Chitosan coatings are an excellent carrier of other functional substances, such as antimicrobials and antioxidants (Dutta et al., 2009; Zhong et al., 2011; Yang et al., 2014).

Aloe vera (*Aloe barbadensis* Miller) is a member of the family Liliaceae. It is one of the most biologically active plants, since it is a rich source of antimicrobial and antioxidant agents, such as phenolic compounds (Vega-Gálvez et al., 2011). Therefore, *A. vera* is widely used in food, pharmaceutical and cosmetic industries (Choi and Chung, 2003; Rodriguez et al., 2010; Vega-Gálvez et al., 2011). The main feature of the *A. vera* fractions (pulp and/or liquid fraction) is their high water content (above 90%), having a complex chemical composition. Some compounds in *A. vera* have been identified as bioactive, such as carbohydrate polymers (mostly acemannan), soluble sugars, organic acids, fibers, proteins, phenolic compounds, vitamins, minerals, aminoacids and mineral salts (Lee et al., 2001; Boudreau and Beland, 2006). Recent studies have demonstrated the effectiveness of *A. vera* extracts (pulp and/or liquid fraction) against numerous forms of diseases in fruits and vegetables caused by fungi (Saks and Barkai-Golan, 1995; Jasso de Rodríguez et al., 2005; Castillo et al., 2010). The main reason to separate the two fractions is due to the difference in bioactive compounds (and concentration) present in each fraction (Jasso de Rodríguez et al., 2005), and thus their biological activity can be different. Recently, coatings based on *A. vera* pulp have been applied on fruits to maintain quality and reduce microorganism proliferation of strawberries and table grapes (Martínez-Romero et al., 2006; Castillo et al., 2010; Guillén et al., 2013). However, as far as we know there is no studies on the application of *A. vera* liquid fraction as coatings on fruits or incorporated in polysaccharide coatings such as chitosan.

The objectives of this work were: (1) to evaluate antifungal and antioxidant activities in vitro of *A. vera* fractions (pulp and liquid), (2) to choose the best chitosan-based formulation to be applied on blueberries, and (3) to evaluate the postharvest quality of cold-stored blueberries coated with chitosan-based coating containing *A. vera* fractions.

2. Materials and methods

2.1. Material

Chitosan was obtained from Golden-Shell Co., China (90% deacetylation). L(+)-Lactic acid 90% and Tween 80 were obtained from Acros Organics (Belgium); ethanol absolute from Chem-lab NV (Belgium). Glycerol (86–89%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and buthylatedhydroxyanisole (BHA) were purchased from Sigma (USA).

Blueberries (*V. corymbosum* L. cv. Duke) were produced in Sever do Vouga (Mirtilusa, Portugal), and harvested in July 2014. The fruits were harvested in the mature state and were evaluated in terms of color, soluble solid content (SSC), citric acid concentration, pH to ensure the same degree of ripeness (results not shown). Blueberries with defects (e.g., cracks) were discarded and only fruit with healthy outer skins and uniform appearance and size were used.

A. vera leaves (*Aloe barbadensis* Miller) (four years old) were provided by *Aloe vera* Ecológico company (Alicante, Spain) in January of 2014. Homogenous leaves were selected according to size, ripeness, color and freshness.

2.2. A. vera *pulp and liquid fractions extraction*

A mechanical procedure was used to obtain the pulp (gel) and liquid fractions according to Jasso de Rodríguez et al. (2005), with some modifications. Firstly, *A. vera* leaves were washed with distilled water and 2% sodium hypochlorite to remove dirt from the surface. Afterward, the whole leaf was weighed and its width, length and thickness were measured. Then, aloin (a yellow-colored liquid) was extracted by cutting the base of the leaves. The skin was carefully separated from the parenchyma using a scalpel-shaped knife, and the epidermis was then separated from the gel using a laboratory roll processor. To complete the extraction, pulp and liquid fraction were separate with a sieve, and pasteurized by heating at 65 °C for 30 min and cooled immediately (procedure repeated three times). The two fractions were stored at −20 °C until further analysis. Table 1 presents the chemical characterization of the *A. vera* fractions performed within our group (Flores-López et al., 2013).

2.2.1. *Antifungal activity in vitro of* A. vera *pulp and liquid fractions*

2.2.1.1. Fungal strains. *B. cinerea* (MUM 10.138), *Penicillium expansum* (MUM 02.14) and *Aspergillus niger* (MUM 92.13) were obtained from MUM (Micoteca da Universidade do Minho, Braga, Portugal). All fungi were routinely cultured at 25 °C for 7–14 d on potato dextrose agar (PDA) (Difco, France), and the spores were collected and diluted with sterile water until suspensions reached a spore concentration of 10^4 mL^{-1}.

2.2.1.2. Antifungal activity assay. Antifungal activity was evaluated following a modification of the procedure reported by Kouassi et al. (2012). 100 μL of fresh pulp or liquid fraction at 0.5%, 5%, 20% and 100% (v/v) was pipetted into a sterile 96-well microplate. The concentrations used for analysis were based on other works where, *A. vera* was used as bioactive compound against microbial contamination (Martínez-Romero et al., 2006; Benítez et al., 2013; Oliveira et al., 2014). Each well was inoculated with a 100 μL aliquot of fungal inoculum to reach a final volume of 200 μL. A positive control was carried out by mixing 100 μL of sterile potato dextrose broth (PDB) (Liofilchem, Italy) with 100 μL of each fungal suspension. The negative control of each group of replicates was a non-inoculated medium. Fungal growth was monitored

Table 1
Chemical characterization of pulp and liquid fraction of *Aloe vera* (results are expressed as % of dry matter basis).

	Pulp (%)	Liquid (%)
Total solids	1.38 ± 0.36	0.65 ± 0.01
Total carbohydrates	33.94 ± 1.73	26.97 ± 0.18
Protein	3.17 ± 0.12	3.28 ± 0.18
Lipids	0.66 ± 0.03	0.53 ± 0.10
Organic acids	22.18 ± 3.27	27.51 ± 2.54
Ashes	0.43 ± 0.06	0.70 ± 0.00
Total phenolic content	1.91 ± 0.09	4.33 ± 0.17

Adapted from Flores-López et al. (2013).

spectrophotometrically at 530 nm (BiotekSinergy II, USA) by measuring optical density (OD) during 72 h (at 24 h intervals) and incubation at 25 °C. Percentage of growth inhibition was determined using the following equation:

$$\text{Inhibition}\,(\%) = \frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}})}{(\text{OD}_{\text{control}})} \times 100 \quad (1)$$

where $\text{OD}_{\text{sample}}$ represents the optical density of the each treatment and $\text{OD}_{\text{control}}$ represents the optical density of the control. Experiments were replicated three times for each mold.

2.2.2. Antioxidant activity assay

Radical scavenging activity of the *A. vera* fractions was measured by DPPH test according with the method of Blois (1958), with some modifications (Rufino et al., 2007; Pinheiro et al., 2015). BHA was used as reference antioxidant and ethanol was used as control. Briefly, 0.2 mL of ethanol and 0.3 mL of the sample dissolved in ethanol (containing 0.1–10 g L^{-1}) were mixed with 2.5 mL of DPPH (60×10^{-6} mol L^{-1} in ethanol) to achieve a final volume of 3.0 mL. The solution was mixed in a vortex and kept at room temperature for 30 min in the dark. Then, 0.2 mL of each sample was transferred into a 96-well microplate to measure absorbance at 515 nm (BiotekSinergy II, USA) and the activity was expressed as percentage of radical scavenging activity (% RSA) relative to the control, using the following equation:

$$\text{RSA}(\%) = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \times 100 \quad (2)$$

where $\text{Abs}_{\text{sample}}$ represents the absorbance of the sample solution and $\text{Abs}_{\text{control}}$ represents the absorbance of the control. IC_{50} value was determined as the concentration of the compound that caused 50% of RSA. All experiments were conducted in triplicate.

2.3. Coating and films preparation

Coating formulations were prepared under a completely randomized design with factorial arrangement, where factors, A = chitosan concentrations of: 0.5, 1.0 and 1.5% (w/v); B = glycerol concentrations of: 0.5, 1.0 and 1.5% (v/v); C = Tween 80 concentrations of: 0, 0.1 and 0.2% (w/v) were studied (Table 2). The concentrations were chosen based on preliminary tests (data not shown). Coating solutions were prepared by dissolving the chitosan in lactic acid (1.0% (v/v)) under agitation during 10 h at 20 °C, to obtain a homogeneous solution. Then, Tween 80 was added as a surfactant, glycerol was added as plasticizer and *A. vera* fraction (liquid or pulp) as antioxidant and antimicrobial agent, with agitation during 3 h at room temperature, to reach complete dissolution. For blueberry shelf-life tests, liquid fraction at 0.5% (v/v) was used.

To prepare the films, a constant amount (28 mL) of coating-forming solution was cast in a Petri dish, with 9.6 cm diameter, to maintain film thickness. The Petri dishes were placed in an oven at 35 °C during 20 h. Films were subsequently stored at 20 °C and 50% of relative humidity (RH) in a desiccator containing Mg $(NO_3)_2{\cdot}6H_2O$ saturated solution at least 48 h, in order to perform water vapor permeability and thickness measurements.

2.4. Coating/film characterization

2.4.1. Wettability

In order to obtain a uniform spreading on blueberry surface, spreading coefficient (W_s) (Eq. (3)) need to be determined. The wettability of the blueberry surface was determined for each coating formulation, and thus the best formulation was selected.

$$W_s = W_a - W_c \quad (3)$$

While the forces of adhesion (expressed as the work of adhesion, W_a) (Eqs. (3) and (4)) favor the spreading of liquid (i.e., chitosan based formulations) on the solid surface (i.e., blueberry), the forces of cohesion (expressed as work of cohesion, W_c) (Eqs. (3) and (5)) promote their contraction.

$$W_a = \gamma_L(1 + \cos(\theta)) \quad (4)$$

Table 2
Spreading coefficient (W_s) achieved for the tested chitosan solutions on blueberry surface.

Formulation	Chitosan (% w/v)	Glycerol (% v/v)	Tween 80 (% w/v)	W_s
1	0.5	0.5	0	-89.79 ± 2.02^{f}
2	0.5	0.5	0.1	-46.61 ± 4.27^{a}
3	0.5	0.5	0.2	-46.12 ± 4.88^{a}
4	0.5	1.0	0	-63.22 ± 4.56^{d}
5	0.5	1.0	0.1	-46.71 ± 4.42^{a}
6	0.5	1.0	0.2	-46.92 ± 4.44^{a}
7	0.5	1.5	0	-84.93 ± 4.9^{ef}
8	0.5	1.5	0.1	-53.05 ± 4.13^{bc}
9	0.5	1.5	0.2	-52.08 ± 2.96^{bc}
10	1.0	0.5	0	-81.15 ± 5.2^{e}
11	1.0	0.5	0.1	-51.85 ± 3.75^{b}
12	1.0	0.5	0.2	-50.63 ± 3.80^{b}
13	1.0	1.0	0	-80.63 ± 3.1^{e}
14	1.0	1.0	0.1	-55.73 ± 2.96^{c}
15	1.0	1.0	0.2	-53.71 ± 3.28^{bc}
16	1.0	1.5	0	-82.97 ± 5.9^{ef}
17	1.0	1.5	0.1	-55.08 ± 3.98^{bc}
18	1.0	1.5	0.2	-53.62 ± 3.28^{bc}
19	1.5	0.5	0	-65.04 ± 6.14^{d}
20	1.5	0.5	0.1	-50.41 ± 4.76^{ab}
21	1.5	0.5	0.2	-52.04 ± 4.91^{bc}
22	1.5	1.0	0	-82.07 ± 2.9^{e}
23	1.5	1.0	0.1	-62.59 ± 2.26^{d}
24	1.5	1.0	0.2	-60.02 ± 1.97^{cd}
25	1.5	1.5	0	-80.32 ± 5.0^{e}
26	1.5	1.5	0.1	-52.70 ± 4.20^{bc}
27	1.5	1.5	0.2	-52.91 ± 4.21^{bc}

[a–f]Different letters in the same column correspond to statistically different samples for a 95% confidence level.

$$W_c = 2\gamma_L \quad (5)$$

To obtain W_s, contact angle (θ) and surface tension (γ_L) were determined. The contact angle at the blueberry surface was measured by the sessile drop method, and observed with a contact angle meter (OCA 20, Dataphysics, Germany). The samples of the coating solution with different concentrations of their constituents (Table 2) were taken with an automatic piston 500 μL syringe (Hamilton, Switzerland) with a 0.75 mm diameter needle. The contact angle at the blueberry surface was measured, using computer aided image processing using a digital camera. To avoid changes on the blueberry, measurements were made in less than 45 s. The surface tension of the coating solution was measured by the pendant drop method using the Laplace–Young approximation (Casariego et al., 2008).

Twenty replicates of contact angle measurements and four replicates of surface tension measurements were obtained at 22 ± 1.8 °C, for each formulation.

2.4.2. Water vapor permeability (WVP)

The WVP of chitosan films was determined gravimetrically, using ASTM E96-92 procedure, with some modifications (Bourtoom and Chinnan, 2008). The permeation cell was filled with 50 mL of distilled water in order to generate a 100% RH (2337 Pa vapor pressure at 20 °C) and the film was sealed on the top of the cells. Then, the cells were weighted using an analytical balance (Mettler AE200) and placed inside a desiccator containing silica (0% RH; 0 Pa water vapor pressure; the air circulation kept constant by using a fan inside the desiccator). The tests were conducted in triplicate and changes in weight of the cells were recorded at intervals of 2 h to record moisture loss over time until steady state was reached. The WVP (kg Pa^{-1} m^{-1} s^{-1}) of the films tested was determined by the following equation:

$$\mathrm{WVP} = \frac{\mathrm{WVTR} \times X}{\Delta P} \quad (6)$$

where WVTR = water vapor transmission rate (kg m^{-1} s^{-1}) through the film calculated from the slope of the curve divided by the film area; X = film thickness (m); ΔP = partial vapor pressure difference (Pa) across the two sides of the film.

Five thickness measurements were randomly taken on each testing sample at different points with a digital micrometer (No. 293-5, Mitutoyo, Japan). Mean values were used to calculate WVP.

2.5. Blueberry shelf-life analyses

2.5.1. Experimental design

The shelf-life analyzes were performed in two main sets of experiments at the same temperature (5 ± 0.6 °C) and relative humidity (90 ± 3%) storage conditions, achieved inside a refrigerated cold room. During storage, samples were placed in aluminum foil containers which were left open inside the controlled temperature and humidity room. Set 1 was composed of non-inoculated blueberries. Three different treatments were tested on set 1: uncoated blueberries (B); chitosan-coated blueberries (BC), and chitosan–*A. vera* liquid fraction coated blueberries (BCA). Set 2 was composed with artificially inoculated (*B. cinerea*) blueberries. Two different treatments were tested on set 2: uncoated blueberries (BF), and chitosan–*A. vera* liquid fraction coated blueberries (BAF). *B. cinerea* was chosen for fruit inoculation, since this is one of the most representative fungi responsible for decay of blueberries (Yang et al., 2014). During shelf-life studies, temperature and relative humidity were recorded with an iButton data logger (Thermochron, USA). Set 1 samples were analyzed microbiologically and physico-chemically at regular intervals (0, 2, 4, 6, 9, 12, 15, 18, 21 and 25 d). Set 2 samples were analyzed during 15 d (0, 2, 4, 6, 9, 12 and 15 d) under the conditions described. The second set was studied in shorter time, since the incorporation of the fungi accelerated the blueberries deterioration.

2.5.2. Coating application and blueberry inoculation

Blueberries were selected based on their ripeness degree and their surface was cleaned. Blueberries samples (average height = 1.28 ± 0.08 cm; average diameter = 1.52 ± 0.1 cm; average weight = 1.58 ± 0.08 g) were dipped into the coating solution (0.5% (w/v) chitosan; 0.5% (v/v) glycerol; 0.1% (w/v) Tween 80 and 0.5% (v/v) *A. vera* liquid fraction) for 5 min and dried in a container with ventilation during 4–5 h at 20 °C to ensure coating dryness. BF and BAF samples were inoculated in the furrow (diametrically opposite to apex), with 10 μL of *B. cinerea* at a spore concentration of 10^4 mL^{-1}.

2.5.3. Physicochemical analyses

2.5.3.1. Titratable acidity (TA), pH, soluble solid content (SSC). The blueberries from each treatment (30 g) were ground in a blender. Titratable acidity (TA) was determined using 942.15 AOAC methods (AOAC, 1997), specific for fruit derivates, by measuring the amount of 0.1 mol L^{-1} NaOH. Results were expressed as % (grams of citric acid equivalent per 100 g of blueberry).

pH measurement it is an important parameter in determining potential microbial growth that could cause deterioration. The pH value was determined using a pH meter (Hanna Instruments Inc., Romania). After the homogenization of the samples, pH was measured by direct immersion of the electrode.

Juice from the fruit was used to determine the soluble solid concentration (SSC) using refractometer RHB-32ATC, previously standardized with water (Hanna Instruments Inc., Romania), according to 932.12 AOAC method (AOAC, 1997). Results were expressed as %.

At each sampling time, and for all physicochemical tests, three samples per treatment were analyzed.

2.5.3.2. Weight loss. Weight loss was evaluated according to Duan et al. (2011) by weighting all samples with a precision balance (Mettler AE200) at the beginning of storage (day 0) and at all sampling days. The percentage of weight loss was determined by the following equation:

$$\text{Weight loss}(\%) = \frac{W_i - W_f}{W_i} \times 100 \quad (7)$$

where w_i is the initial sample weight and w_f is the sample weight.

2.5.4. Microbiological analyses

Microbiological analyses were determined using 966.23 AOAC method (AOAC, 1997). Blueberry samples (20 g) of each treatment were transferred to individual sterile stomacher bags with 180 mL of 0.1% peptone water (Becton, Dickinson and Company, France). The samples were homogenized in a blender Stomacher 3500 (Seward Medical, London, U.K.) for 2 min. Samples were prepared in triplicate. Serial decimal dilutions were carried out, and 100 μL was spread on PDA. Plates were incubated during 5 d at 25 °C and total molds/yeast colony forming units (CFU) were determined. Microbiological counts were converted to log CFU g^{-1}, and the means and standard deviations were calculated.

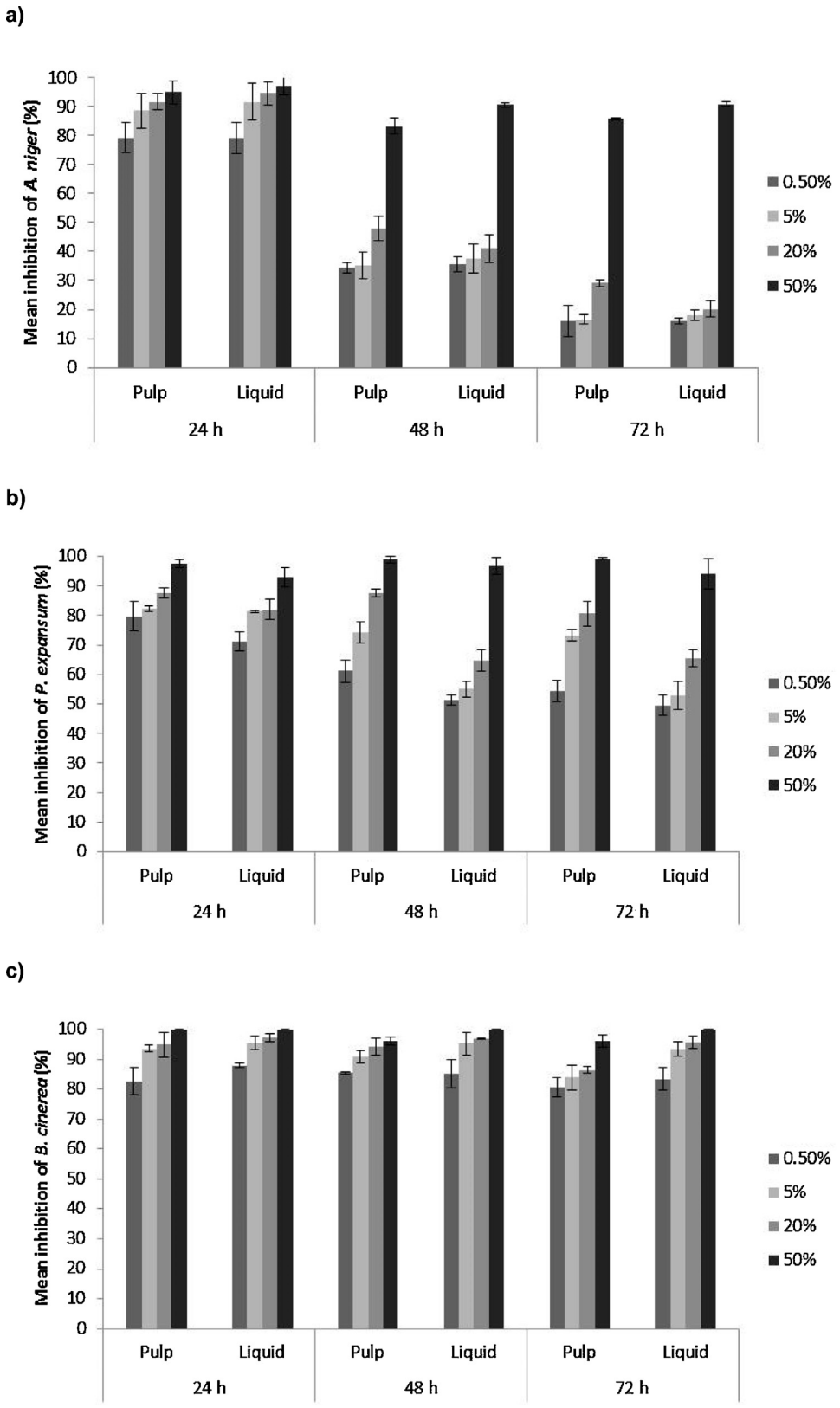

Fig. 1. Mean inhibition (%) effect of *A. vera* liquid and pulp fractions at different concentrations on (a) *A. niger*, (b) *P. expansum* and (c) *B. cinerea* growth.

2.6. Statistical analyses

The data were analyzed using Sigma Plot 11 and Microsoft Windows Excel 2010 software. Data were subjected to analysis of variance (ANOVA) ($p < 0.05$) and the mean comparisons were performed using the Tukey's HSD test to examine if differences between treatments and storage time were significant ($\alpha = 0.05$).

3. Results and discussion

3.1. Wettability of chitosan-based solutions on blueberry surface

In practical terms, the closer the W_s values are to zero, the better a surface will be coated (Martins et al., 2010). The results shown that depending on the amount of chitosan, glycerol and Tween 80 added, W_s values are statistically different ($p < 0.05$) (Table 2). The addition of Tween 80 (surfactant) reduced cohesion forces, therefore reducing the surface tension and increasing the wettability. Thus, this surfactant improved compatibility between the solution and the fruit skin surface. The results shown that the surfactant improves wetting properties, since W_s values of solutions with different concentrations of Tween 80 (0.1 and 0.2%) are the nearest from 0, probably due to the reduction of surface tension of the liquid (Table 2). The improvement of W_s with addition of Tween 80 was also shown by Cerqueira et al. (2009), when studying wettability of chitosan-based coating on the surface of cheese. As could be seen in Table 2, 0.5% glycerol provided good wettability. The addition of glycerol was important to reduce the stiffness of the film, preventing an easier breaking (Olivas and Barbosa-Cánovas, 2005). The formulations with better values of W_s (closer to zero) were the ones with lower chitosan and glycerol concentrations, in the presence of Tween 80. Considering the solutions tested, coating solutions that presented best values of W_s were solutions 2, 3, 5 and 6. However, there are no significant differences ($p > 0.05$) between these four coating formulations. Once there were no statistically significant differences, it has been assumed that their differentiation must be made on the basis of other criteria, such as WVP, since water loss is a major problem in the deterioration of the blueberry fruit.

3.2. Water vapor permeability (WVP)

The four coatings formulations with best wettability values were chosen for the WVP evaluation. The incorporation of glycerol (Gly) and Tween 80 (Twe80) at different concentrations into chitosan (Chi) films did not change significant WVP values ($p > 0.05$) for formulations 2 ($(5.1 \pm 0.22) \times 10^{-13}$ kg Pa^{-1} m^{-1} s^{-1}), 3 ($(5.2 \pm 0.2) \times 10^{-13}$ kg Pa^{-1} m^{-1} s^{-1}) and 5 ($(5.4 \pm 0.11) \times 10^{-13}$ kg Pa^{-1} m^{-1} s^{-1}). Formulation 6 (0.5% Chi + 1.0% Gly + 0.2% Twe80) presented higher WVP values ($(5.8 \pm 0.078) \times 10^{-13}$ kg Pa^{-1} m^{-1} s^{-1}) than the other films ($p < 0.05$), which means that film 6 presented a poor barrier to water vapor. This behavior can be related to an addition of a higher concentration of glycerol, which can change the polymer network and create mobile regions with greater interchain distances, thus increasing the permeability of the films (Cerqueira et al., 2012). The WVP values of the films were between 5.1×10^{-13} and 5.8×10^{-13} kg Pa^{-1} m^{-1} s^{-1}. This WVP values are comparable with those reported by Garcia et al. (2006) for starch-based films (1.77×10^{-13} kg Pa^{-1} m^{-1} s^{-1}) and Hambleton et al. (2008) for ι-carrageenan ($1.18–23.5 \times 10^{-13}$ kg Pa^{-1} m^{-1} s^{-1}).

Since no significant differences were found between formulation 2, 3 and 5, the formulation with lower concentrations of each constituent was chosen (formulation 2—0.5% Chi + 0.5% Gly + 0.1% Twe80), thereby benefiting the use of less compounds concentration, reducing the costs of this packaging. Once selected the best formulation according to wettability and WVP values, the following step was to determine the most suitable *A. vera* fraction (liquid or pulp) to be incorporated in chitosan-based films, according to antifungal and antioxidant activities (Section 3.3).

3.3. Antifungal and antioxidant activities of A. vera fractions

Pulp and liquid fractions exhibited similar inhibitory results for the three fungi studied (Fig. 1). Overall, *B. cinerea* and *P. expansum* presented more growth inhibition in the presence of *A. vera* pulp or liquid than *A. niger* (Fig. 1a–c). Antifungal activity of *A. vera* can be referred to the presence of bioactive compounds, such as quinones and phenol compounds (flavonoids), which can be more active against some fungi than others (Sarabia et al., 1999). Castillo et al. (2010) reported different antifungal behavior when different fungi were exposed to *A. vera*. Also, these authors reported that *A. vera* gel inhibited mycelium growth of *P. digitatum* and *B. cinerea*. However, *P. digitatum* presented higher growth inhibition than *B. cinerea*.

After 72 h of incubation with 0.5% *A. vera* liquid, an inhibition of 84.86 ± 1.44% was observed for *B. cinerea*, the main mold causing blueberry deterioration (Fig. 1c), while *P. expansum* and *A. niger* presented 49.59 ± 8.56% and 15.91 ± 0.94% of growth inhibition, respectively (Fig. 1a and b). With 0.5% *A. vera* pulp, an inhibition of 80.57 ± 2.36% was observed for *B. cinerea*, an inhibition of 54.51 ± 6.83% for *P. expansum* and 15.95 ± 5.45% of growth inhibition for *A. niger*. It can be seen in Fig. 1, that 100% of each *A. vera* fractions were not a source of growth compounds for the three fungi studied, since growth was not observed over time (72 h). The mechanism of inhibition of *A. vera* has been associated with the ability to suppress germination and mycelial growth inhibition due to the presence of more than one bioactive component (Nabigol and Asghari, 2013). As can be seen in Table 1, the total phenolic content (identified in the literature as antifungal and highly antioxidant) is higher in the liquid fraction than in the pulp fraction (4.33 ± 0.17% and 1.91 ± 0.09%, respectively). Saks and Barkai-Golan (1995) found that *A. vera* pulp presented antifungal activity against some fungi, such as *B. cinerea* and *P. expansum*, and the inhibitory results are similar to those obtained in our study (Fig. 1).

According to Table 3, the liquid fraction has a higher antioxidant activity ($p < 0.05$) than pulp (40.86 ± 2.72% and 30.52 ± 1.15% RSA, respectively) and, consequently, liquid fraction had the lowest values of IC_{50} (7.76 ± 0.89 g L^{-1}). This result can be related to the total phenolic compounds higher content present in liquid fraction (Table 1). Previous works have reported the direct relationship of scavenging activity with the concentration of phenolic and flavonoid content in *A. vera* skin, pulp and their ethanolic extracts (Cíz et al., 2010; Moniruzzaman et al., 2012). Also, Hu et al. (2003) reported high antioxidant activity for ethanolic extracts of *A. vera* and concluded that *A. vera* at various development stages contains different active components (polysaccharides and flavonoids) and consequently, different antioxidant activity. These authors

Table 3
Radical scavenging activity (RSA) and IC_{50} of pulp and liquid fraction of *Aloe vera*. RSA and IC_{50} values for BHA are given for comparison.

Sample	RSA (%)*	IC_{50} (g L^{-1})
Pulp	30.52 ± 1.15^{a}	22.37 ± 0.81^{a}
Liquid	40.86 ± 2.72^{b}	7.76 ± 0.89^{b}
BHA	82.26 ± 1.56^{c}	0.07 ± 0.01^{c}

[a–c]Different letters in the same column correspond to statistically different samples for a 95% confidence level.
* % RSA of pulp, liquid and BHA corresponds to 10, 6 and 0.5 g L^{-1}, respectively.

reported different RSA values, according to the age of the leaves of 62.70 ± 0.44% (two years), 72.19 ± 0.98% (three years) and 67.64 ± 2.99% (four years).

As far as we know, our study is the first to show that *A. vera* fractions (pulp and liquid) have different antioxidant activity and that the liquid fraction presents the highest antioxidant capacity (Table 3). Since the liquid fraction obtained a higher antioxidant activity and a lower IC_{50} than the pulp at concentrations of 6 g L^{-1} (corresponding to 0.6%), and considering that optimal antifungal activity and film forming ability were achieved at 0.5%, liquid fraction at 0.5% was selected to be incorporated into the chitosan films for subsequent surface application on blueberries. In order to confirm that the incorporation of *A. vera* fractions in the coating formulations did not change their properties (wettability, WVP), test were performed and no statistically significant differences were observed between coating formulations with and without *A. vera* (results not shown).

3.4. Shelf-life properties

3.4.1. Physico-chemical analyses

In general, TA values decreased over time (Fig. 2), probably due to the use of organic acids (such as citric acid) in the respiration process (Gol et al., 2013). In set 1, B samples had significantly lower TA values than BC and BCA after 9 d of storage; thus, coating helped retain TA of blueberries ($p < 0.05$). In set 2, there was no significant difference ($p > 0.05$) in the TA values of BF and BAF at 15th day. However, TA values of BF sample decreased abruptly (0.51–0.16%), when compared to other treatments. This can be explained by an increase of fungal population, once citric acid can be considered as source of compounds for their growth. BAF treatment prevented rapid TA decrease from 0 to 9 d of storage as compared with the BF treatment, and remained constant during storage time. These results are in agreement with those reported by Benítez et al. (2013), that demonstrated that kiwifruit coated with *A. vera* reduced TA values over storage time.

Coatings maintained pH values of blueberries at lower levels compared to the uncoated (non-inoculated and inoculated) fruit samples, over 9 d ($p < 0.05$) (Table 4). BCA samples maintained lower pH value (3.10 ± 0.010) during 12 d of refrigeration, compared to other samples. Also, Benítez et al. (2013) showed that kiwifruit coated with *A. vera* slightly lower the initial pH, and maintained pH values over 12 d. After 12 d of storage, pH values of uncoated (non-inoculated and inoculated) blueberries were significantly ($p < 0.05$) higher than initial pH (day 0) (Table 4). BAF treatment was able to maintain for approximately 15 d, the initial pH value (3.09 ± 0.015), while the BF treatment had higher pH values. Thus, chitosan–*A. vera* coated samples attenuated pH changes despite inoculation of blueberries. In general, increase of pH values may be related to blueberry spoilage, with formation of alkaline autolysis compounds (e.g., nitrogenous compounds) (Soares et al., 2013) and production of fungal metabolites, once pH changed when CFU g^{-1} values increased (Fig. 5).

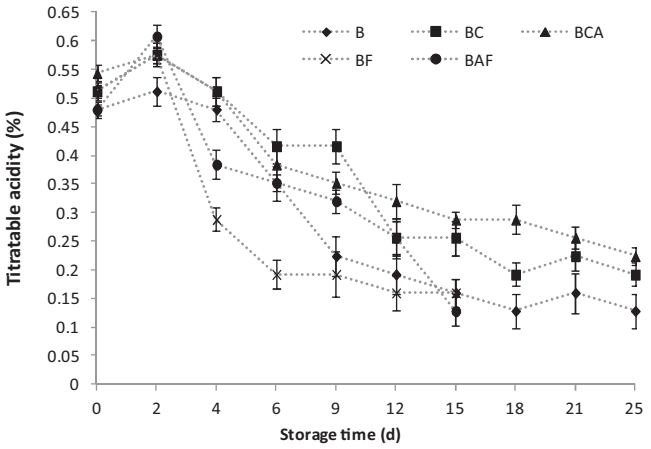

Fig. 2. Titratable acidity (%) of blueberries throughout storage time for non-inoculated B, BC and BCA samples, and inoculated BF and BAF samples. Error bars represent the standard deviation. B—non-inoculated uncoated blueberry samples; BC—non-inoculated chitosan-coated samples; BCA—non-inoculated chitosan–*A. vera* coated samples; BF—inoculated uncoated samples; BAF—inoculated chitosan–*A. vera* coated samples.

SSC was relatively stable during blueberry storage but some differences can be seen between different treatments (Fig. 3). BC and BCA treatments were those with minor SSC variation compared to uncoated fruit (B), since from day 15, the values increase considerably (Fig. 3a). It can be observed an increase of SSC from 15th until 25th day for B samples. This is probably due to water loss, which caused increase of sugar concentration. BC presented a low level of the initial SSC (10.87 ± 0.10%), and maintained this value constant until the end of 25 d (11.57 ± 0.40%), having a more drastic increase at 15th to 18th day (10.87 ± 0.25% to 11.97 ± 0.21%). BAF treatment maintains SSC values constant until day 12 (11.10 ± 0.27 to 11.30 ± 0.10%) (Fig. 3b). The uncoated sample (BF) shown SSC values with changes (ranging from 10.57 ± 0.61 to 11.9 ± 0.44%), probably due to the fact that the fungus had consumed more sugars (such as glucose) at day 4 (10.57 ± 0.61). However, at the end of 15 d, significant differences were not seen between the BF and BAF treatments (Fig. 3b). Martínez-Romero et al. (2006) also showed that cherries coated with *A. vera* pulp maintains SSC over time.

Table 4
pH values of blueberry samples during 25 d of storage at 5 °C.

Storage time (d)	Treatment				
	B	BC	BCA	BF	BFA
0	3.38 ± 0.010[a]	3.12 ± 0.015[bc]	3.15 ± 0.021[b]	3.29 ± 0.010[d]	3.09 ± 0.015[c]
2	3.32 ± 0.010[a]	3.14 ± 0.021[b]	3.09 ± 0.015[bc]	3.36 ± 0.031[a]	3.08 ± 0.020[c]
4	3.41 ± 0.012[a]	3.16 ± 0.015[b]	3.14 ± 0.020[b]	3.47 ± 0.021[c]	3.08 ± 0.006[d]
6	3.41 ± 0.006[a]	3.14 ± 0.010[b]	3.13 ± 0.025[bc]	3.51 ± 0.010[d]	3.09 ± 0.025[c]
9	3.27 ± 0.020[a]	3.09 ± 0.015[b]	3.11 ± 0.015[b]	3.04 ± 0.177[b]	3.10 ± 0.020[b]
12	3.54 ± 0.015[a]	3.34 ± 0.031[b]	3.10 ± 0.010[c]	3.56 ± 0.030[a]	3.22 ± 0.045[d]
15	3.52 ± 0.010[a]	3.36 ± 0.020[b]	3.28 ± 0.021[c]	3.52 ± 0.020[a]	3.13 ± 0.040[d]
18	3.58 ± 0.044[a]	3.35 ± 0.128[b]	3.27 ± 0.032[b]	–	–
21	3.56 ± 0.010[a]	3.39 ± 0.055[b]	3.31 ± 0.067[b]	–	–
25	3.82 ± 0.046[a]	3.53 ± 0.020[b]	3.43 ± 0.020[c]	–	–

[a–d]Different letters in the same column correspond to statistically different samples for a 95% confidence level. B, non-inoculated uncoated blueberry samples; BC, non-inoculated chitosan-coated samples; BCA, non-inoculated chitosan–*A. vera* coated samples; BF, inoculated uncoated samples; BAF, inoculated chitosan–*A. vera* coated samples. Values reported are the means ± standard deviations.

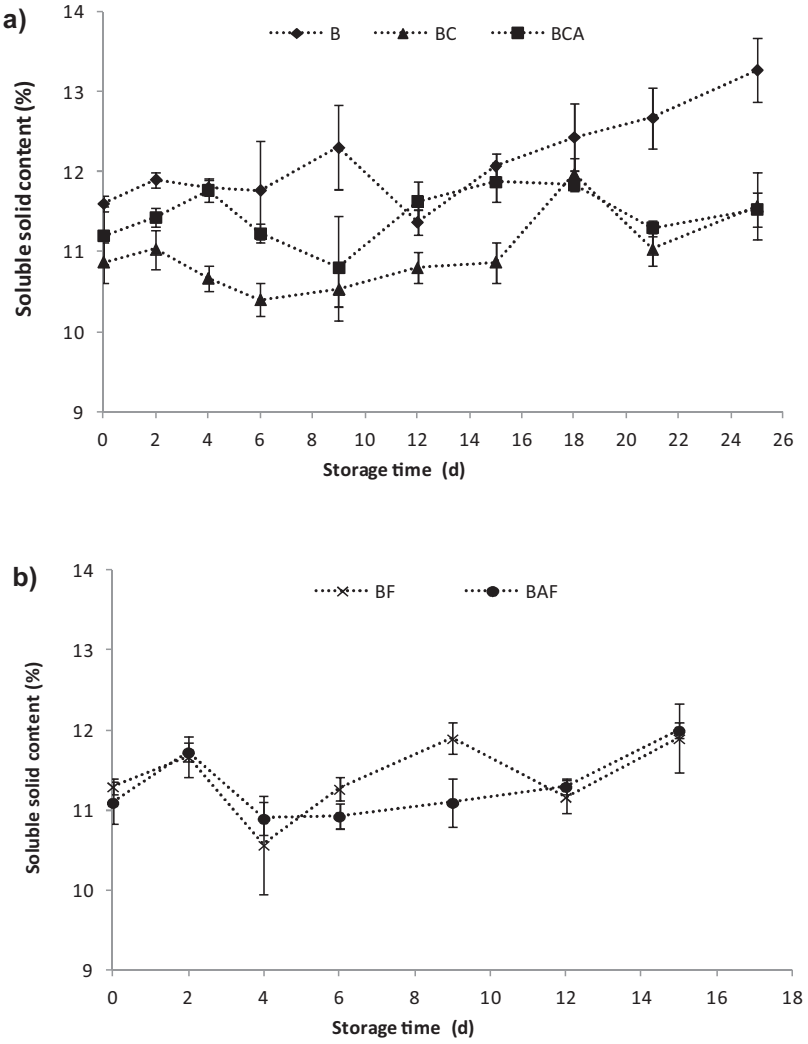

Fig. 3. Soluble solids content (SSC), expressed as a %, of blueberries throughout storage time for (a) non-inoculated B, BC and BCA samples, and (b) inoculated BF and BAF samples. Error bars represent the standard deviation.

It can be considered that weight loss corresponds nearly exclusively to water loss since other components that can be lost (aromas, flavors, and gases product of respiration) are residual in terms of weight variation (Olivas and Barbosa-Cánovas, 2005). BCA treatment delayed dehydration of fruit, since the lowest weight loss (i.e. 3.7% after 25 d) was obtained for this treatment (Fig. 4). At 25th day, control sample (B) weight loss is 1.67 times higher than BCA treatment. As to BC, it was observed that weight loss decreases around 1% comparing to B sample (6.2%). Possibly, hydrophobic compounds present in *A. vera* fraction created a more efficient moisture barrier. Martínez-Romero et al. (2006) showed that *A. vera* pulp films retarded water loss and controlled gas exchange, thereby reducing respiration and oxidation reactions, during the storage period. For inoculated samples (BF and BAF), weight loss was more noticeable on 15th day of storage. This behavior may be due to exposure to more adverse conditions (e.g., a sharp increase of microbial contamination, as can be seen in Fig. 5b), which may cause a greater loss of water and therefore, a greater weight loss.

Hereupon, these results shown that higher SSC observed in B samples could be due to sugar concentration as a result of water loss by dehydration. Water loss caused an apparent increase on the concentration of SSC that may be incorrectly interpreted as a true

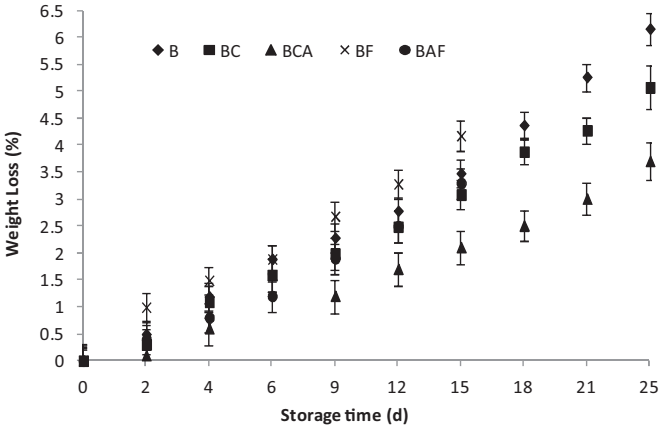

Fig. 4. Blueberries weight loss (%) throughout storage time for non-inoculated B, BC and BCA samples, and inoculated BF and BAF samples. Error bars represent the standard deviation.

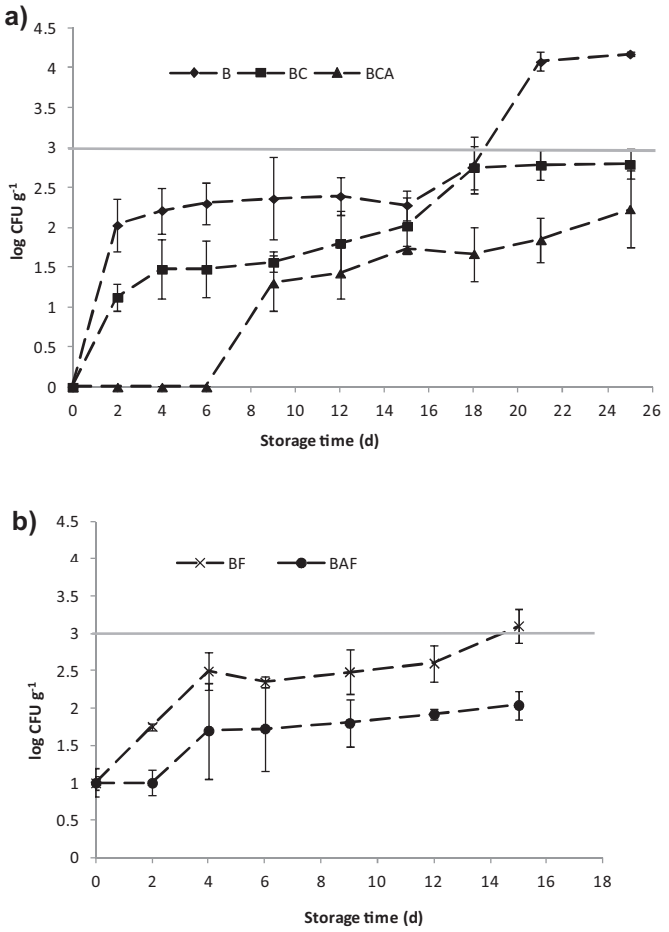

Fig. 5. Total yeasts and molds counts (log CFU g^{-1}) of blueberries throughout storage time for (a) non-inoculated B, BC and BCA samples, and (b) inoculated BF and BAF samples. The maximum permissible limit for consumption is 3.0 log CFU g^{-1} ($\log(CFU)_{max}$), represented by horizontal grey line.

change in the amount of acids or sugars present on fruits (Olivas and Barbosa-Cánovas, 2005). Also, the lower weight loss found in chitosan–*A. vera* coated blueberries can be translated by the maintenance of soluble solids over time. Thus, it was inferred that during postharvest storage, acid metabolism converted starch and acid to sugar, thus resulting in the decrease of TA values and increase of SSC values (Duan et al., 2011).

3.4.2. Microbiological analyses

Microbiological results showed significant differences between samples ($p < 0.05$). The treatment that showed best results was BCA, once this treatment inhibited the growth of *B. cinerea* during 6 d (Fig. 5a). Also, this treatment presented log CFU g^{-1} values below the values observed for uncoated fruit (B) and BC samples from 9th day until 25th day of storage. These results showed that antifungal activity of *A. vera* liquid fraction demonstrated in vitro was also manifested on blueberry fruit, which may be associated with fungicide action of the *A. vera* (Jasso de Rodríguez et al., 2005; Martínez-Romero et al., 2006). BC showed lower CFU g^{-1} values (<3 log CFU g^{-1}) when compared to B during 15 d of storage, a fact that may be associated with antifungal action of chitosan (Cerqueira et al., 2011; Ai et al., 2012; Ruiz-Navajas et al., 2013; Qiu et al., 2014). Regarding inoculated blueberries samples, results showed fungistatic effect of BAF during the storage period (Fig. 5b). This coating (chitosan + *A. vera*) may improve blueberry safety through inhibition or delay of microbial growth. Chitosan coating and chitosan–*A. vera* coating were effective on extending blueberry shelf life, as can be seen in Fig. 5, since at day 15, BF treatment exceeds 3 log CFU g^{-1} (considered as the upper acceptability limit for microbiological quality of raw fruits, in Portugal) (Santos et al., 2005). The differences between BC and BCA ($p < 0.05$) at the end of 25 d (2.8 ± 0.19 and 2.2 ± 0.47 log CFU g^{-1}, respectively) may be due to combination of chitosan and *A. vera*, resulting in better antifungal capacity. Castillo et al. (2010) also showed that *A. vera* pulp could be applied as a postharvest treatment to inhibit microbial (yeasts and molds) spoilage and reduce decay incidence during storage of table grapes.

Visual evaluation confirmed that the uncoated blueberries had extensive mould growth on surface after 25 d of storage (Fig. 6). The chitosan-coating solution with or without *A. vera* liquid fraction appears to have inhibited the growth of moulds, when compared with uncoated blueberries samples.

4. Conclusions

The combination of chitosan and *A. vera* fractions as edible coating materials has great potential in expanding the shelf-life of blueberries. *A. vera* liquid fraction showed higher total phenolic content and antioxidant activity, as well as lower values of IC_{50} than pulp fraction. The application of an edible coating containing *A. vera* on the blueberry surface after harvesting provides an additional barrier to reduce postharvest contamination by fungi, and also by reducing the rate of water loss, the two main factors of blueberry quality loss in postharvest. The best edible coating was formulated with chitosan–*A. vera* liquid fraction at 0.5%, that can extend the shelf life for about 5 d, which represent a significant commercial value to blueberries producers.

The incorporation of antifungal compounds, such as *A. vera*, into edible films or coatings provides a novel way to improve safety and shelf-life of blueberries, without using synthetic compounds. *A. vera* fractions could be an attractive natural alternative against fungi that attack fruits and vegetables, avoiding excessive use of chemicals, and thereby contributing to prevent the occurrence of health and environmental problems.

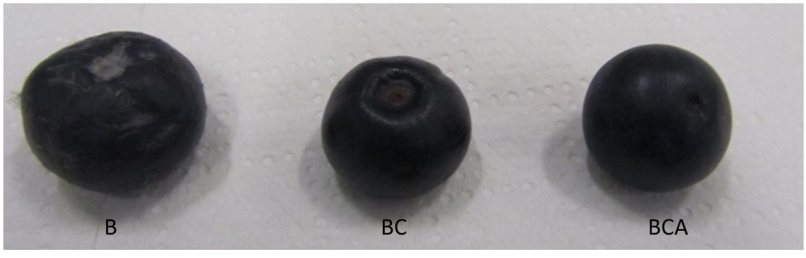

Fig. 6. Uncoated blueberry samples (B), chitosan-based coated samples (BC), and chitosan–*A. vera* coated samples (BCA) on the 25th day of storage at 5 °C.

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