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

Jorge M. Vieira[a], María L. Flores-López[b], Diana Jasso de Rodríguez[c], Maria C. Sousa[a,d], António A. Vicente[b], Joana T. Martins[b,*]

[a] Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, Coimbra, Portugal
[b] CEB—Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[c] Universidad Autonoma Agraria Antonio Narro, Calzada Antonio Narro No. 1923, Colonia Buenavista, 25315 Saltillo, Coahuila, Mexico
[d] CNC—Centro de Neurociências e Biologia Celular, Universidade de Coimbra, Portugal

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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

* Corresponding author. Fax: +351 253 604 429.
E-mail address: joanamartins@deb.uminho.pt (J.T. Martins).

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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).