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ORIGINAL ARTICLE

An approach to improve the safety and quality of ready-to-eat blueberries

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Abstract

Bioactive edible coatings were developed and applied to blueberries as a natural treatment. *E. coli* O157:H7, *L. innocua*, *S. aureus*, and *P. aeruginosa* were subjected to four bioactive compounds and to three film-forming solutions (FFS). Vanillin and geraniol at low concentrations (1.2–1.8 mg/mL and 0.4–1 μL/mL) demonstrated significant inhibitory effects on all pathogens counts. Chitosan (Ch) showed a high antimicrobial activity (final counts below 2 log CFU/mL). The effectiveness of Ch plus vanillin (Ch-Va) and geraniol (Ch-Ge) in improving the safety were tested against pathogens inoculated on blueberries. Ch, Ch-Va, and Ch-Ge coatings exerted a bactericidal effect on all pathogens (from 1.24 to more than 2 log reductions). Significant reduction in yeast and mold counts was achieved with Ch (1.09 log) and Ch + Va (1.74 log). Sensory attributes of blueberries remained acceptable. Ch-Va and Ch-Ge were an alternative to improve the quality and safety and could be effective in extending the shelf life of ready-to-eat blueberries.

Practical applications

The present study proposes the use of natural coatings enriched with biopreservatives as a technological alternative to enhance the quality and safety of minimally processed fruits. According to the results obtained, the application of chitosan plus vanillin/geraniol coatings on fresh blueberries would allow to offer a safe product and respond the growing demand of consumers for fresh, environmentally friendly and chemical preservatives-free foods. These findings and those obtained by the sensory evaluation support the practical application of this alternative in the minimally processed fruit industry.

1 | INTRODUCTION

The development of safe fruits is one of the key drivers for the food industry due to an increasing demand for naturally preserved foods. Blueberries (*Vaccinium spp.*) are among the most popular berries in retail markets and are sold in fresh, frozen, and processed forms for various food applications (Cantín et al., 2012). However, fresh berry fruit deteriorates rapidly due to water loss, juice leakage (stem scar injury), and gray mold. Fruit decay in blueberries is usually caused by fungi. The growth of *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, and *Listeria monocytogenes* on blueberries has been reported by different authors (Miller, Rigdon, Robinson, Hedberg, & Smith, 2013; Popa, Hanson, Todd, Schilder, & Ryser, 2007; Sun et al., 2014).

Several preservation technologies, including cold storage, UV, irradiation, modified atmosphere, and ozonation, have been used to reduce deterioration, prolong shelf life, and retain the nutritional value of fresh blueberries (Chiabrando, Peano, Beccaro, Bounous, & Rolle, 2006; Trigo et al., 2006). In addition, edible coatings have been studied for extending shelf life of some fresh berry fruits (Ribeiro, Vicente, Teixeira, & Miranda, 2007; Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2006). The development of edible coatings as carriers of active ingredients (such as natural antimicrobials) is considered a promising packaging alternative to maintain freshness of fresh-cut fruits (Dhall, 2013). Gellan, secreted by the bacterium *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*) and alginate, extracted from marine brown algae (Phaeophyceae) are common polysaccharides used as gelling agent in food industry. Also, the

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antimicrobial effectiveness of chitosan polysaccharide has been tested in a wide range of food products like meat, fish (Ceylan, Sengor, & Yilmaz, 2018) and nowadays it has taken on enormous importance in the control of postharvest pathogenic microorganisms. Previous studies have demonstrated that chitosan coatings can effectively decrease mold growth, thus extending shelf-life of strawberries and blueberries (Duan, Wu, Strik, & Zhao, 2011; Park, Stan, Daeschel, & Zhao, 2005). Vanillin is the major constituent of vanilla beans and is used as a flavoring/aroma compound in foods and fragrance industries. It is known to be antimycotic and bacteriostatic (Cassani, Tomadoni, Viacava, Ponce, & Moreira, 2016; Tomadoni, Cassani, Moreira, & Ponce, 2015). Another natural occurring antimicrobial agents are resveratrol (3, 4′, 5 Trihydroxy-trans-stilbene), pomegranate dried extract (*Punica granatum* L.) and geraniol. This last is a common constituent of several essential oils, emitted from the flowers of many species and it is present in vegetative tissues of many herbs (Chen & Viljoen, 2010). Its potential as an antimicrobial agent has been highlighted in several studies (Chen & Viljoen, 2010; Tomadoni et al., 2015). In this way, chitosan, gellan, and alginate coatings can be proposed as vehicles of biopreservatives to ensure the microbial safety of ready-to-eat blueberries.

Therefore, the objective of this work was to study the effectiveness of chitosan-biopreservatives coatings in preserving the quality and safety of fresh blueberries. Also, the effect of these coatings on the survival of *Escherichia coli* O157:H7, *Listeria innocua*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* inoculated in blueberries was studied. Moreover, since that the organoleptically acceptable concentration depends on each individual biopreservative, the specific food matrix, the application method, and its subsequent handling until consumption (Ghabraie, Vu, Tata, Salmieri, & Lacroix, 2016) sensory acceptance by consumers was also considered in this work.

2 | MATERIALS AND METHODS

2.1 | Plant material

Blueberries (*Vaccinium corymbosum* L.) cv. Snow Chaser were purchased from a local producer in Concordia, Entre Rios province (Argentina). Blueberries at commercial maturity (pH 2.84 ± 0.03; 12.35 ± 0.07 °Brix; $1.20 \pm 0.09\%$ titrable acidity) were selected based on their uniformity of size and color. Blueberries were washed with 0.5% citric acid solution for 30 s, drained and air-dried for 30 min prior to treatments application.

2.2 | Culture maintenance and inoculum preparation

Nontoxigenic *Escherichia coli* O157:H7 FP605/03, *Listeria innocua* CIP 8011, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used in this study. All microbial strains were grown for 24 hr at 37°C in Luria Bertani (LB) broth. Then, 100 μL of each culture were transferred to 9.9 mL of LB broth at 37°C for 24 hr twice before each experiment. Each culture was properly diluted in sterile peptone solution until achieving the desired concentration (10^4–10^5 CFU/mL).

2.3 | Bioactive compounds

The bioactive compounds (BC) used in this work were: vanillin (Va, Firmenich SAICYF, Argentina), geraniol (Ge, Firmenich SAICYF, Argentina), pomegranate dried extract (Pg, PureBulk, USA; 35% ellagic acid, 19% gallic acid, 10% punicalagin A, 5% punicalagin B, 2% caffeic acid), and resveratrol (Res, Sigma). Dimethyl sulfoxide (DMSO) and Tween 80 were used to enhance dissolution properties.

2.4 | Preparation of FFS

Medium molecular weight chitosan (Ch, ACOFAR, Mar del Plata, Argentina; 98% deacetylation degree, 0.7% ash, 46 cP viscosity), food-grade gellan gum (GG, Kelcogel®, CPKelco, Chicago, IL) and food grade sodium alginate (SA, Keltone LV, ISP, San Diego, CA) were used. SA (2% w/v) or GG (0.5% w/v) powders were dissolved into distilled water by gently stirring at 70°C until the solution became clear (Rojas-Grau, Tapia, & Martın-Belloso, 2008). Ch solutions were prepared by dispersing chitosan powder (1.5% w/v) in 0.7% lactic acid solution with magnetic stirring at 23°C, the pH was adjusted to 5.6 according to Goñi, Tomadoni, Roura, and Moreira (2017).

2.5 | *In vitro* assay: Antimicrobial effectiveness of BC and FFS

2.5.1 | Sensitivity assay

The sensitivity of each pathogen to different bioactive compounds was performed by the agar diffusion method on inoculated LB agar plates with holes filled with a specific bioactive compound. Pathogens sensitivity to different biopreservatives was classified by the diameter of inhibition halos according to Alvarez, Ponce, and Moreira (2013). Each assay was performed in duplicate on three separate experimental runs.

2.5.2 | Determination of minimum inhibitory concentrations and minimum bactericidal concentrations

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were obtained by Broth microdilution method in sterile 96-well microplates. The concentration ranges used were: 0.5–3.9 mg/mL for Va, 0.3–3.06 μL/mL for Ge, 0.5–4 mg/mL for Res, and 1.5–7 mg/mL for Pg. The microplates were incubated at 37°C for 24 hr.

MCBs were determined by spreading on the surface plating on LB agar 0.1 mL of the first well with no visible growth of each assessed compound and the next concentrations (Alvarez et al., 2013). The plates were incubated at 37°C for 24 hr and the numbers of colonies were determined. Microbial counts were expressed as log CFU/mL. Each assay was performed in duplicate on three separate experimental runs.

2.5.3 | Tube-assay method

Film-forming solutions were evaluated by the tube-assay method according to Moreira, Pereda, Marcovich, and Roura (2011). Test tubes containing LB broth, pathogen with and without FFS were incubated at 37°C for 24 hr. At that time, 100 mL of each test tube were

plated on LB agar and incubated at 37°C for 24 hr. The effect of film-forming solutions was obtained comparing the counts of control sample and treated samples. Each assay was performed in duplicate on three separate experimental runs.

2.6 | *In vivo* assay: Antimicrobial effectiveness of Ch-edible coating plus BC on blueberries

2.6.1 | Fruit inoculation

Blueberries were inoculated by spraying with a specific pathogen suspension before coating, according to Moral, Bouhmidi, and Trapero (2008). After inoculation, the fruit was dried for 30 min at 25°C before coating. To evaluate native microflora behavior, a noninoculated control sample was handled as described above but sprayed with sterile water.

2.6.2 | Coating application

Inoculated and noninoculated blueberries where immersed into the different coating solutions for 2 min at 20°C, and then they were left dripped for 1 min. Coated fruit was left drying in biosecurity cabin by air at 25°C for 30 min before assays Romanazzi, Gabler, Margosan, Mackey, & Smilanick, 2009).

After being treated, blueberries (with or without the pathogen inoculation) were placed into 300 mL polypropylene containers with caps. Microbial counts and sensory analysis were performed in duplicate from three containers. The assays were carried out on three independent experimental runs.

2.6.3 | Microbial studies

Microbial populations were evaluated as follow: 10 g of fruit were placed in a sterile plastic bag with 90 mL of peptone water and were homogenized with a Stomacher 400 Circulator Homogenizer (pH 7.2). Serial dilutions (1:10) of each homogenized sample were made in the same diluents until surface spreading. Mesophilic aerobic bacteria (MES) were incubated and enumerated on Plate Count Agar at 37°C for 48 hr; yeasts and molds on Yeast Glucose Chloramphenicol at 25°C for 5 days; *E. coli* O157:H7 on Eosin Methylene Blue at 37°C for 48 hr; *L. innocua* on CHROMagar Listeria at 37°C for 48 hr; *S. aureus* on Baird Parker supplemented with sodium tellurite and egg yolk at 37°C for 48 hr and *P. aeruginosa* on Cetrimide Agar at 37°C for 48 hr. Microbial counts were expressed as log CFU/g (Alvarez et al., 2013). Analyses were performed from three samples containers and two replicate counts were carried out for each container.

2.6.4 | Sensory evaluation

To evaluate the impact of the treatments on sensory attributes of noninoculated ready-to-eat blueberries, a qualitative descriptive analysis was carried out according to Alvarez, Ponce, Mazzucotelli, and Moreira (2014) with some modifications. Briefly, a panel comprised of ten members from the Faculty of Food Science (UNER); aged 25–50 years trained for this task and with experience in blueberries sensory evaluations, carried out the sensorial assay. The attributes evaluated were overall visual quality (OVQ), odd-odor, odd-flavor, and firmness (measured by a deformation test, squeezing the fruit between the forefinger and the thumb). Every sample was presented with a three-digit code, randomly and one at time to a panelist singly. Water was provided for rinsing between samples. Each attribute was quantified by it intensity on a 5 cm unstructured intensity scale. OVQ was scored from 0 (*rare appearance*) to 5 (*fresh appearance*), odd-odor from 0 (*intense odd-odors*) to 5 (*lack of odd-odors*), odd-flavor from 0 (*intense odd-flavor*) to 5 (*typical-lack of odd-flavor*), and firmness from 0 (*soft*) to 5 (*firm*).

In addition, a sensory acceptability test of odor and flavor was performed by a group of 60 individuals who regularly consumed blueberries. Acceptability of treated and untreated blueberries was evaluated using a hedonic scale (1–10, *disliking* to *liking*). Results were expressed as general odor and flavor acceptability of consumers to the product (Ayala-Zavala et al., 2013). The limit of acceptance for both sensorial analyses was 50% of the scale (Alvarez et al., 2014).

2.7 | Statistical analysis

This study was performed based on a completely random design. For each assay, three independent runs with at least two repetitions of each parameter were performed. Results informed in this work are presented as mean values accompanied by their standard errors. Analysis of variance (ANOVA) was performed ($p \leq 0.05$) to estimate significant differences between treatments and means were compared using Tukey test at a significance level of $p \leq 0.05$ with MINITAB Statistical Software, Release 16 for Windows, Minitab Inc., State College, PA.

3 | RESULTS AND DISCUSSION

3.1 | Sensitivity assay

Antimicrobial inhibition zones for BC against *E. coli*, *L. innocua*, *S. aureus*, and *P. aeruginosa* are shown in Figure 1. The obtained results indicated that Res produce 20–22 mm in diameter inhibition zones for *E. coli*, *L. innocua*, and *S. aureus*. Therefore, *E. coli* and *L. innocua* were shown as extremely sensitive to Res (40 mg/mL). *S. aureus* resulted very sensitive. Nevertheless, *P. aeruginosa* was not sensitive to this compound. *E. coli* and *L. innocua* were significantly more sensitive to Res than other bioactive compounds. Moreover, *E. coli* showed a lower susceptibility to Va and Ge, with 15–18 mm diameter inhibition halos. Instead, *L. innocua* does not present significant differences between the rest of compounds (Va, Ge, and Pg). For *S. aureus* the best results were obtained with Res and Pg, significantly different from the results obtained with Ge and Va. *P. aeruginosa* presented the lowest sensitivity.

3.2 | Determination of minimum inhibitory concentrations and minimum bactericidal concentrations

Table 1 summarized the obtained MICs and MBCs for different biopreservatives against the four bacterial strains by broth microdilution method. Among the natural compounds, Ge was found to be the most effective one, with the lowest MIC values of 0.4, 0.7, 0.6, and 1 μL/mL against *E. coli*, *L. innocua*, *S. aureus*, and *P. aeruginosa*, respectively,

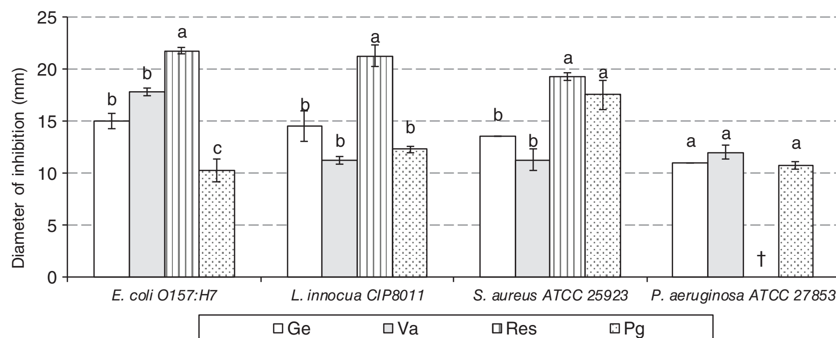

FIGURE 1 Sensitivity of gram negative and gram positive pathogenic bacteria against to geraniol (Ge), vanillin (Va), resveratrol (Res), and pomegranate extract (Pg) biopreservatives by the agar diffusion method.The diameter of each hole (6 mm) is included. The sensitivity to the different antimicrobial agents was classified by the diameter of the inhibition halos as not sensitive, diameters = <8 mm; sensitive, diameters = 9–14 mm; very sensitive, diameters = 15–19 mm; and extremely sensitive, diameters = >20 mm. For each microbial population, columns with different letters are significantly different ($p < 0.05$). †No differences with control (DMSO)

and followed by Va (except for *L. innocua*, in which case it was followed by Res). Several *in vitro* studies were performed using Va and Ge against *E. coli* and *L. monocytogenes* as indicators (Cava-Roda, Taboada-Rodríguez, Valverde-Franco, & Marin-Iniesta, 2012; Tomadoni et al., 2015). They reported similar values with those presented in our study. The MIC of Pg could only be obtained for *S. aureus* in the range of evaluated values. In addition, it could not be possible to determine the Res MIC for *P. aeruginosa*, which was the microorganism that presented the highest MIC values and therefore the highest resistance. This fact was in agreement with the agar diffusion method results mentioned above (Figure 1). Regarding MBCs values, once again Ge is highlighted as the most effective one for all microorganisms assayed. Va MBC value for *E. coli*, as well as Res and Pg MCB values for all pathogens studied could not be determined. In according, Ferreira and Domingues (2016) and Qin et al. (2014) did not find significant antimicrobial effects of Res and Pg.

As a rule, the results confirm that the antibacterial activity of each bioactive compound is in relation to the concentration used. Moreover, it has to be considered that slight differences in the applied methods for determination of antimicrobial activity combined with the use of different microorganisms and strains could explain the different MIC reported by several authors. Furthermore, it is known that a strain cannot represent the behavior of a species (Borges, Ferreira, Saavedra, & Simoes, 2013). This interpretation can also help to clarify the discrepancy between results of sensitivity assay (which is a qualitative assay that groups result in ranges) and broth dilution method (which is a quantitative method that reports a concentration of each compound with activity on a microorganism).

3.3 | Film-forming solutions

The low diffusivity of biopolymers in agar diffusion method is well known, thus it becomes necessary to evaluate the inhibitory effects of FFS by a more appropriate method, like tube-assay method (Moreira et al., 2011). Figure 2 shows the inhibitory effects of Ch, GG, and SA film-forming solutions after 24 hr of incubation at 37°C. On the one hand, neither GG nor SA exerted antimicrobial effect on pathogens tested. On the other hand, all pathogens strains were significantly inhibited by Ch solution. In fact, the degree of reduction exceeded the detection limit of the method (<2 log) for *E. coli*, *L. innocua*, and *P. aeruginosa*. For *S. aureus* the reduction reached 4 log CFU/mL compared with the growth of control sample.

In spite of the general affirmation of a role for cell wall Gram type on response to chitosan antimicrobial efficacy, there are authors that have found better results for gram-positive bacteria than for negative-bacteria, and these findings are supported by several *in vitro* assays (Goy, De Britto, & Assis, 2009). Fernandes et al. (2008) detected about 3 log reduction on *E. coli* counts with 0.5% w/v chitosan at 4 hr, and at the same concentration a bactericidal effect on *S. aureus* independently of molecular weight of polymer. Eaton, Fernandes, Pereira, Pintado, and Malcata (2008) observed reductions of about 4 log when they evaluated chitosan (0.5% w/v) as a film-forming solution against both *E. coli* and *S. aureus* after 24 hr of incubation. These authors

TABLE 1 *In vitro* antimicrobial activity of geraniol (Ge), vanillin (Va), resveratrol (Res), and pomegranate extract (Pg) against gram negative and gram positive pathogenic bacteria: MIC and MBC values

	MIC				MBC			
Microbial population	Ge (μL/mL)	Va (mg/mL)	Res (mg/mL)	Pg (mg/mL)	Ge (μL/mL)	Va (mg/mL)	Res (mg/mL)	Pg (mg/mL)
E. coli O157:H7	0.4	1.2	3.4	>7	0.5	>3.9	>4	>7
L. innocua CIP 8011	0.7	1.2	1.1	>7	0.8	3.9	>4	>7
S. aureus ATCC 25923	0.6	1.8	3.4	4.5	0.7	3.9	>4	>7
P. aeruginosa ATCC 27853	1	1.8	>4	>7	3.6	3.6	>4	>7

Each assay was performed in duplicate on three separate experimental runs.

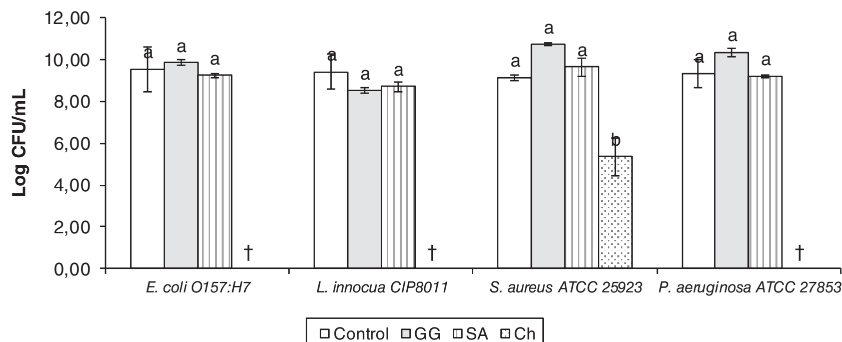

FIGURE 2 Effects of chitosan (Ch), gellan gum (GG), and sodium alginate (SA) film-forming solutions against gram negative and gram positive pathogenic bacteria by tube assay method. For each microbial population, columns with different letters are significantly different ($p < 0.05$). †Values below the detection limit of the method

concluded that even though chitosan exerted a high antimicrobial effect on both gram-negative and gram-positive microorganisms, the mode of action was different and strongly dependent on polymer molecular weight and the target microorganism.

3.4 | *In vivo* assay: Microbiological quality

To evaluate the effectiveness of each treatment against all indicator bacteria in the same conditions, it was agreed to use the higher MIC found between pathogens. Therefore, chitosan-forming solution plus vanillin and geraniol (at 1 MIC) were proposed for *in vivo* assay. In this way, Table 2 detailed the microbiological results obtained when blueberries were treated as follows: chitosan coating (Ch), chitosan + vanillin (Ch-Va), chitosan + geraniol (Ch-Ge) and an uncoated sample as control (C).

As shown in Table 2, neither treatment was able to achieve significant initial reductions in mesophilic aerobic microorganism (MES). However, significant reductions were obtained in yeasts and molds population with Ch and Ch-Va treatments.

In agreement with our results, Bezerra de Aquino, Fitzgerald Blank, Cristina, and Lins de Aquino Santana (2015) found no significant reductions in MES counts determined in guavas when they were treated with chitosan–cassava starch coatings at 1 day of storage. Moreover, significant reductions in yeasts and molds population were reported for this author. Similarly, Santos Frazao, Fitzgerald Blank, and Lins de Aquino Santana (2017) studied edible coating of cassava starch, chitosan, and *Myrcia ovata* Cambessedes essential oils on mangaba fruit finding the same results. On the contrary, Alvarez, Ponce, and Moreira (2018) found significant initial reduction in MES (1.7 log CFU/g) when fresh blueberries were treated with chitosan coating (2% w/v), but no significant initial reduction in yeasts and molds population.

Regarding inoculated blueberries, all treatments exerted significant inhibitory effects on all pathogens tested, showing a bactericidal effect. Besides, some of treatments achieved great reductions that left pathogen below the detection limit. Particularly, *E. coli* counts decreased from 3.59 to 2.35 log CFU/g when fruit was coated with Ch. No detectable growth of this pathogen was determined when blueberries were treated with Ch-Va or Ch-Ge coatings. *L. innocua* was only found in Ch-Va coatings, showing a reduction of 2.28 log compared to control. For *S. aureus*, reductions of 2.8 and 1.88 log were obtained when blueberries were treated with Ch and Ch-Va coating, respectively. Finally, *P. aeruginosa* was only detected in blueberries coated with Ch-Ge, with pathogen counts 1.37 log below control count. The discrepancy between the effectiveness of the applied treatments against each pathogen could be attributed to the concentration used of each biopreservative. It is well known that

TABLE 2 Effect of chitosan-based coatings, and chitosan enriched with vanillin and geraniol on native microflora and pathogens populations of blueberries

Microbial population	Treatment	Log CFU/mg
Mesophilic aerobic bacteria	C	3.22 ± 0.11^a
	Ch	2.74 ± 1.04^a
	Ch-Va	3.04 ± 0.80^a
	Ch-Ge	2.50 ± 0.71^a
Yeast and molds	C	3.74 ± 0.02^a
	Ch	2.65 ± 0.49^{bc}
	Ch-Va	2.00 ± 0.00^c
	Ch-Ge	3.06 ± 0.08^{ab}
E. coli O157:H7	C	3.59 ± 0.16^a
	Ch	2.35 ± 0.06^b
	Ch-Va	$<2^{\dagger}$
	Ch-Ge	$<2^{\dagger}$
L. innocua CIP 8011	C	4.87 ± 0.00^a
	Ch	$<2^{\dagger}$
	Ch-Va	2.59 ± 0.16^b
	Ch-Ge	$<2^{\dagger}$
S. aureus ATCC 25923	C	4.80 ± 0.33^a
	Ch	2.00 ± 0.00^c
	Ch-Va	2.92 ± 0.11^b
	Ch-Ge	$<2^{\dagger}$
P. aeruginosa ATCC 27853	C	4.17 ± 0.11^a
	Ch	$<2^{\dagger}$
	Ch-Va	$<2^{\dagger}$
	Ch-Ge	2.80 ± 0.28^b

Uncoated control (C), chitosan (Ch), Ch plus vanillin (Ch-Va), Ch plus geraniol (Ch-Ge). Data shown are the means ± standard deviation. For each microbial population, treatments were compared. Means followed by different letters are significantly different ($p < .05$).
†Values under the detection limit of the method.

in vitro efficacy of biopreservatives may decrease when tested by *in vivo* assay. According to this, several works support the fact that natural preservatives concentration should be increased when they are applied *in vivo* to reach the same effectiveness as it shows *in vitro* (Alvarez et al., 2014). Nevertheless, higher concentration of this compound could negatively affect sensory quality of products, and the adopted criteria must take it into account. As explained above, biopreservative concentrations used in this work correspond to those which were found for *P. aeruginosa* MIC, because of their higher resistance. Thus, although 1 MIC of Ge was effective on inhibition of *P. aeruginosa in vitro*, higher concentrations could be necessary to obtain the same results in food systems. The same explanation can be used for *L. innocua* resistance to Ch-Va treatment. In the case of *S. aureus*, it was the only microorganism that could be quantified when treated with Ch film-forming solution. Other authors found similar results when they assessed geraniol or vanillin on fruit. About these results, Tomadoni et al. (2015) found a significant reduction of 2.2–2.4 log/mL on *E. coli* O157:H7 counts inoculated in strawberry juice treated with geraniol at 0.6 and 1.2 μL/mL. With respect to vanillin, Rojas-Grau et al. (2007) found that initial counts of *L. innocua* in apple pieces coated with alginate-apple puree containing vanillin (0.3 and 0.6% w/w) decreased approximately 3.0 log cycles during the early hours of storage, with respect to initial levels of inoculum.

Native microflora was more resistant than inoculated pathogens. It could be due to the fact that these populations are well adapted to fruit environment. This fact was observed by others authors like Alvarez et al. (2014), who used bioactive compounds (tea tree essential oil, propolis extract and gallic acid) to control native microflora of fresh-cut mixed vegetables for soup, as well as inoculated *E. coli* O157:H7, reporting less effectiveness in controlling the microbiota than the inoculated pathogen.

3.5 | Sensory evaluation

A qualitative descriptive analysis was carried out for sensory evaluation. The results obtained from 10 judges are shown in Figure 3. Differences of 0.6–0.8 points below were obtained for Overall Visual Quality (OVQ) of treated samples compared to uncoated sample. It could be attributed to the coating impact on distinctive natural looking "bloom" of blueberries (natural waxy coating on untreated fruit), which is a quality parameter of fresh blueberries. Nevertheless, these slight differences resulted not significant in OVQ. Regarding firmness, no differences were detected in coated fruit by the manual test used. Alvarez et al. (2018) found significant differences on firmness values when compared fresh and chitosan coated blueberries by a digital penetrometer at initial time of storage. Furthermore, several works on coated blueberries shows a significant improvement on firmness retention and in some cases even an increase of it when this parameter is evaluated in a certain period of time, at a certain storage temperature, and in general by instrumental techniques (Alvarez et al., 2018; Jiang, Sun, Jia, Wang, & Huang, 2016; Sun et al., 2014). Odd-odor and odd-flavor were the most affected parameters by the biopreservatives. It is well known that the application of natural compounds as biopreservatives could be limited due to the sensory impact they carry on, especially at high concentrations (Goñi et al., 2009). Ch-Va and Ch-Ge samples were shown as significantly different from control and chitosan coated samples. Since odd-odor and odd-flavor were presented to the judges as differences from typical odor and flavor of fresh fruit, the only conclusion that can be drawn is that biopreservatives imparted changes on these attributes that were strongly detected. To find out if those changes were positive or negative, and even more, if the treated blueberries resulted acceptable, an acceptability analysis was carried out.

Figure 4 summarizes the data obtained by the consumers test. Firstly, no sample was below the acceptability limit (50% of scale, 5 points). Despite this, samples treated with Ch-Ge obtained the lowest scores with 6 and 5.9 points for odor and flavor, respectively. Regarding odor acceptability, no significant differences were found between samples. Besides, the highest score was obtained by uncoated sample (C) and even though the fruit was of excellent quality and at commercial maturity, it only reached 7.1 of 10 points of scale. It could be attributed to blueberries not being very aromatic fruits. Regarding flavor, Ch-Ge samples were significantly different from the rest and given that Ch samples were not different from control samples, the negative impact of flavor could be attributed to geraniol. According to this result, Cassani et al. (2016) performed sensory

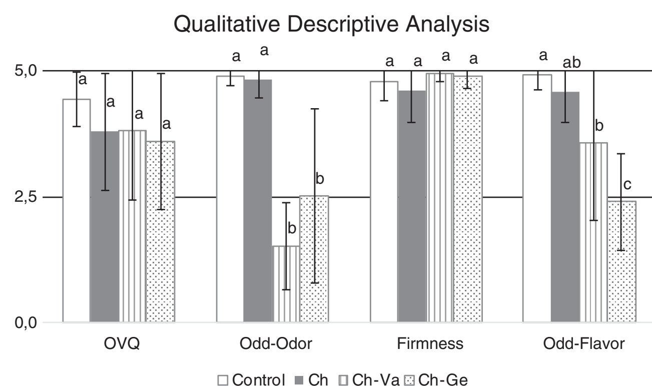

FIGURE 3 Sensory attributes of blueberries as affected by the application of chitosan-based edible coating (Ch) enriched with vanillin (Ch-Va) and geraniol (Ch-Ge) by a qualitative descriptive analysis. For each attribute, columns with different letters represent treatments significantly different ($p < 0.05$)

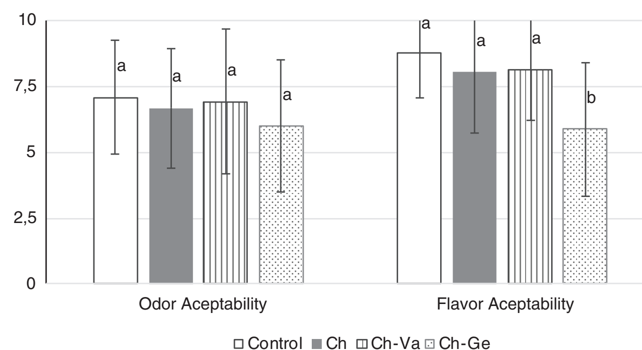

FIGURE 4 Sensory acceptability of blueberries as affected by the application of chitosan-based edible coating (Ch) enriched with vanillin (Ch-Va) and geraniol (Ch-Ge) by a sensory acceptability test. For each attribute, columns with different letters represent treatments significantly different ($p < 0.05$)

evaluation of fiber-enriched strawberry juice and reported that the use of geraniol (0.4 μg/mL) and vanillin (1.8 mg/mL) caused strong changes in those attributes relating with odor and flavor (sweet and acid taste). As regards odor, these authors found that both biopreservatives were above the limit of acceptance immediately after they were applied. Regarding flavor, the sweetness of geraniol treated samples resulted with the most committed attribute and was considered unpleasant by the panelists.

4 | CONCLUSION

Natural compounds that had shown *in vitro* effect were also effectively applied by *in vivo* assay. As regard microbiological quality, all applied treatments were able to exert significant antimicrobial effects on each pathogen tested (with reductions between 35% and higher than 41% in the best results). Even more, they were able to reduce some pathogen counts below detected limit (<2 log), showing a strong bactericidal effect. Also, the applied coatings reduced yeasts and molds, the main spoilage agents of blueberries. Concerning sensory quality, although samples with vanillin or geraniol imparted detectable strong odor and flavor to blueberries, all treatments resulted acceptable.

These results are promising in terms of improving safety and quality of ready-to-eat blueberries by using chitosan edible coatings enriched with vanillin or geraniol. Despite this, further studies are needed to evaluate the impact of these coatings on the evolution of microbial populations and sensory attributes during refrigerated storage, as well as other physicochemical and nutritional parameters.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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