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ORIGINAL ARTICLE

Prebiotic edible coatings with biopreservatives: Quality and safety of fresh apple cubes

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Abstract

Apple fiber (2g/L) was incorporated along with vanillin (2.5g/L) and geraniol (0.6mL/L) into gellan coatings and their effects on the microbiological, physicochemical, nutritional and sensory quality of fresh-cut apple (12 days at 5±1 °C) were studied. Also, the impact of coatings on the evolution of *Escherichia coli* O157:H7 and *Listeria innocua* inoculated on apples was evaluated. Geraniol enhanced antioxidant properties of apples and allowed growth rate reduction of native microbiota. Vanillin minimized color changes, improved firmness, reduced yeasts/molds growth and prolonged sensory shelf-life by 4 days. Moreover, both biopreservatives exerted initial bactericidal effects on *E. coli* and geraniol reduced *E. coli* counts. Also, a bacteriostatic effect against *L. innocua* was found during the first 4 days. Therefore, these functional coatings might be good alternatives for enhancing quality and assuring safety of apples cubes. Vanillin showed the greatest potential to be applied technologically for improving the overall quality of apple cubes.

Practical applications

The use of natural and functional coatings to enhance the quality of minimally processed fruits constitutes a novel technological alternative to respond to the growing rejection of consumers of foods that contain chemical preservatives that could damage their health. Apple fiber enriched-gellan coatings added with vanillin provide a safe product with high nutritional quality and a prolonged shelf life.

1 | INTRODUCTION

Consumers are becoming more aware of the impact of eating habits on health. As a consequence, consumption of fresh fruits and vegetables and functional foods has increased significantly in recent years. A great part of functional foods market in Europe includes foods enriched with prebiotics (Sheehan, Ross, & Fitzgerald, 2007). Apple fiber is a functional food additive due to its prebiotic effect encouraging the growth of healthy bacteria, highlighting the importance of their antioxidant properties and providing additional health-promoting effects (Grigelmo-Miguel & Martín-Belloso, 1998; Marín, Soler-Rivas, Benavente-García, Castillo, & Pérez-Alvarez, 2007).

The development of fresh-cut fruit market is increasing rapidly. These foods are practical and convenient, their quality is similar compared to fresh fruits and do not generate waste when consumed. However, postharvest handling and processing operations such as washing and cutting accelerate microbial and enzymatic spoilage (Moreira, Roura, & Ponce, 2011; Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008). Moreover, fresh-cut fruits could cause outbreaks of foodborne diseases. The survival and growth of pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella*, *and Escherichia coli* on apples has been reported (Abadias, Alegre, Usall, Torres, & Viñas, 2011; Abadias, Usall, Alegre, Torres, & Viñas, 2009; Alegre, Abadias, Anguera, Oliveira, & Viñas, 2010).

In this way, edible coatings constitute safe and effective technologies to improve quality and extend shelf-life of fresh produce. The development of edible films and coatings able to vehiculate bioactive agents (antioxidants, nutrients, probiotics, prebiotics, and natural antimicrobials) is a promising option to enhance quality of fresh-cut fruits and vegetables (Dhall, 2013). Vanillin (4-hydroxy-3-methoxybenzaldehyde), present in vanilla beans, is widely used as flavoring agent. Antioxidant properties of vanillin were demonstrated by its use in foods rich in polyunsaturated fatty acids. Moreover, vanillin resulted effective as antifungal agent when applied in fruit purees and fruit-based agar systems (Fitzgerald,

Stratford, & Narbad, 2003). In addition, geraniol (3,7-dimethylocta-trans-2,6-dien-1-ol) is a monoterpene alcohol naturally present in plant essential oils. Researchers have demonstrated the effectiveness of geraniol as antimicrobial and insect repellent (Chen & Viljoen, 2010). The amount of nutrients and antimicrobials incorporated into films and coatings need to be correctly evaluated taking into account the effects on their basic functionality and sensory acceptability (Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009). The development of health promoting and safe fruits is a challenge for the food industry due to an increasing demand for natural foods enriched with physiologically active components such as prebiotics. In a previous work, Moreira, Cassani, Martín-Belloso, and Soliva-Fortuny (2015) incorporated dietary fibers to edible coating formulations and were able to improve the nutritional value of coated apple cubes without affecting their fresh-like quality attributes. Therefore, the present work proposes the enrichment of gellan-apple fiber coatings with natural compounds as vanillin and geraniol to improve their functionality. The aim was to study the effectiveness of these coating formulations in preserving the quality and safety of fresh-cut "Red delicious" apple. Effects of coating treatments on microbial counts, antioxidant status, color, firmness, and sensory quality of apple cubes were evaluated along 12 days of storage at 5 °C. Also, the impact of these coatings on the survival of *E. coli* O157:H7 and *L. innocua* inoculated in apple cubes was studied.

2 | MATERIALS AND METHODS

2.1 | Materials

"Red delicious" apples were acquired in a local market at commercial maturity and stored at 5 ± 1 °C until processing. Food grade gellan (Kelcogel, CPKelco, Chicago, IL) was the carbohydrate polymer employed to prepare coatings. Apple fiber extract (Indulleida SL, Lleida, Spain) with a purity of 55.90% w/w (13.10 and 42.80% w/w of soluble and insoluble dietetic fiber, respectively) was added to enrich gellan solutions. Vanillin and geraniol (Sigma-Aldrich, Argentina) were used as antimicrobial compounds recognized as GRAS (Generally Recognized as Safe) (Code of Federal Regulations Title 21, FDA).

2.2 | Coating formulations and dipping solutions

Coating forming solution was prepared by dissolving gellan gum in distilled water (5 g/L), heating, and maintaining at 70 °C for 2 hr. The solution was cooled to room temperature and glycerol (6 g/L) was added as plasticizer. Also, apple fiber extract (2 g/L) was incorporated into the gellan solution. Later, vanillin (2.5 g/L) or geraniol (0.6 ml/L) were added to gellan-fiber coating solution as biopreservatives; these concentrations correspond to the minimal inhibitory concentration (MIC) value of each compound according to the results found by Tomadoni, Cassani, Moreira, and Ponce (2015) in in vitro assays. Besides, ascorbic acid (10 g/L) was incorporated into a calcium chloride (20 g/L) bath used to crosslink biopolymers. The concentrations of the ingredients used were selected based on Moreira et al. (2015).

2.3 | Fruit coating

At first, apples were washed, rinsed with tap water, and dried. Afterward, apples were manually peeled, cored, and diced obtaining 1.5 cm-side cubes. To minimize the exposure of apple tissues to air, a maximum of four fruits were cut, and treated simultaneously. The apple pieces were immersed into the different gellan-based coating forming solutions for 2 min. The excess of coating solution was allowed to drip off for 1 min before submerging the fruits again for 2 min in the ascorbic acid-calcium chloride solution. Drying of coated apple cubes was carried out in a biosafety chamber using air at 25 °C for 30 min. Regarding treatments, apple pieces were coated with: gellan-based-solution enriched with apple fiber (GF), gellan-based-solution enriched with apple fiber plus geraniol (GF + ge), and gellan-based-solution enriched with apple fiber plus vanillin (GF + va). Fresh control samples (C) dipped into ascorbic acid-calcium chloride solution but not into the coating forming solution were prepared as a reference.

After treatments, 50 g of fruit were packed in 200-ml polypropylene containers (thickness 0.4 mm, Cotnyl, Buenos Aires, Argentina). Containers were covered with a polyolefine film (PD 960, Sealed air CRYOVAC, Argentina) with an O_2 permeability of 7,000 ml/m^{2}d and CO_2 permeability of 20,000 ml/m^{2}d, sealed using a horizontal thermo-sealing machine for plastic bags (HL, FS-300, Argentina) and stored at 5 ± 1 °C and 95% of relative humidity. Two containers (experimental units) of each treatment were randomly removed from storage at each sampling time (Day 0 immediately after treatment application, 4, 8, and 12 days of storage) and used for analyses.

2.4 | Evolution of quality attributes during storage

In the first study, different quality attributes of fresh-cut apple treated with gellan-biopreservative coatings (enriched with apple fiber) were monitored throughout 12 days of cold storage.

2.4.1 | Microbiological analysis

Native microflora counts (mesophilic bacteria and yeast and molds) on apple cubes were determined during storage. Samples of 10 g of fruit (taken from five different pieces) were separated from each container, placed in sterile bags, diluted with 90 ml of 1 g/L aqueous solution of peptone and treated in a stomacher blender during 2 min. Plate Count Agar (PCA) and chloramphenicol glucose agar (CGA; Britania, Argentina) plates were seeded using serial dilutions of the homogenates. Colony counts were made on PCA plates after 48 hr of incubation at 30 °C to determine mesophilic bacteria and CGA plates after 3–5 days at 25 °C for yeast and molds. Results were expressed as $\log_{10}$ CFU/g. Analyses were carried out at Days 0, 4, 8, and 12 using two containers per treatment and sampling time; counts were made by duplicate for each container.

Microbial growth curves were obtained for mesophilic bacteria and yeast and molds of samples subjected to different treatments. Baranyi function (Baranyi, Roberts, & McClure, 1993) was used for modeling experimental data. Kinetic parameters, including initial count, maximum specific growth rate, and maximum count attained in the stationary phase for each curve, were determined from the

obtained Baranyi values. Also, the coefficient of determination R^2 was informed. In all cases, no lag phase Baranyi models were used for modeling due to a better fit observed.

2.4.2 | Determination of antioxidant properties

Extraction of antioxidants

A 10 g apple sample was taken from each randomly sampled container and homogenized 2 min with 20 ml of 80% ethanol. This mixture was treated in a sonicator for 30 min and centrifuged at 14,000 rpm for 15 min at 4 °C. Subsequently, the supernatant was separated and filtered. The obtained extracts were used for determinations of total phenolic content (TPC) and DPPH antioxidant capacity.

Total phenolic content

TPC was quantified colorimetrically following the methodology described by Viacava, Roura, and Agüero (2015) with some modifications. 0.2 ml of each adequately diluted extract was added to 1 ml of 1:10 diluted Folin Ciocalteu reagent. This mixture was allowed to stand 3 min at room temperature. Subsequently, 0.8 ml of 7.5% Na_2CO_3 (Biopack, Argentina) solution was added to the mixture followed by a 2 hr incubation period at room temperature. Absorbance measurements were made at 765 nm in a UV–VIS spectrophotometer (1601 PC UV–VIS, Shimadzu Corporation, Kyoto, Japan). A standard curve of gallic acid was utilized to calculate TPC and results were registered as mg gallic acid equivalents (GAE)/ 100 g of fresh weight. Measurements were made by triplicate.

DPPH antioxidant capacity

The free radical scavenging activity of apple extracts on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was assessed following the method proposed by Viacava et al. (2015). At first, a 100 μM DPPH solution was prepared using DPPH (Sigma-Aldrich, Argentina) and ethanol as solvent. Then, 100 μl of ethanol was added to 3.9 ml of 100 μM DPPH to measure the initial absorbance of the DPPH solution. Then, 100 μl of each adequately diluted extract was allowed to react with 3.9 ml of 100 μM DPPH solution. Reaction tubes were incubated for 1 hr at room temperature in the darkness. Absorbance measurements were made at 517 nm and reductions in DPPH solution absorbance due to reaction with the extracts were calculated. Finally, the antioxidant capacity was shown as DPPH inhibition percentage. Measurements were made by triplicate.

2.4.3 | Color and firmness measurements

Color of apple pieces was determined using a colorimeter (Lovibond, RT Series, London, England). Color was registered by the CIE - $L^*a^*b^*$ uniform color space (CIE, 1978). The impact of treatments on surface color of apple cubes was assessed using parameters such as lightness (L^*) and hue degree (h° = arctan [b^*/a^*]). Ten fruit pieces taken at random from each pair of sampled containers at each sampling time were subjected to color analyses. Measurements of color parameters were registered by duplicate for each piece.

Fruit firmness was measured with a digital penetrometer (FHT-801, Test Equipment Depot, Melrose MA) using a 3.5 mm plunger diameter. Maximum strength (N/cm^2) required to penetrate the apple piece was registered. Ten pieces taken at random from two containers for each treatment and sampling time were used for firmness determinations.

2.4.4 | Sensory analysis

Sensory quality of coated and uncoated fresh-cut apple was assessed along cold storage following the methodology described by Alvarez, Ponce, and Moreira (2018). Briefly, eight members of the UNMdP Food Engineering Research Group (25–50 years) experienced in fruit and vegetable sensory analysis performed the evaluation of apple samples. Samples were qualified according to their overall visual quality (OVQ), color, flavor, and odor. Attributes were assessed using an unstructured intensity scale of 5 cm. Scores for OVQ ranged from 0 (highly spoiled appearance) to 5 (fresh appearance); flavor from 0 (intense odd flavor) to 5 (typical-no odd flavor); and odor from 0 (intense off-odors) to 5 (fresh-like odor). The acceptability limit was 2.5 (50% of the scale).

2.5 | Effect of coatings on inoculated *E. coli* O157:H7 and *L. innocua*

In a second study, fresh-cut apple was inoculated with *E. coli* O157:H7 and *L. innocua* to simulate a contamination due to inadequate manipulation of the fruit during postharvest. Then, inoculated apple cubes were coated with gellan-biopreservatives solutions, packaged, and stored during 12 days at 5 °C. Microbial count evolution of both pathogen indicators along storage was studied.

2.5.1 | Culture and inoculum management

Non-toxigenic *E. coli* O157:H7 FP605/03 provided by Malbran Institute (Buenos Aires, Argentina) and *L. innocua* CIP8011 (Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina) were utilized. Particularly, *L. innocua* was selected as *L. monocytogenes* biological indicator due to its similar susceptibility to physical, chemical, or thermal procedures. Strains were grown at 35–37 °C in brain-heart infusion (BHI) broth (Britania Lab, Buenos Aires, Argentina) during 24 hr. Before each assay, 100 μl of each culture were added to BHI broth (9.9 ml) and incubated 24 hr at 35–37 °C. This procedure was repeated twice consecutively to achieve cells in stationary growth phase. Afterward, 10 ml of each culture previously prepared were mixed with 90 ml of a 1 g/L sterile peptone solution to obtain *E. coli* and *L. innocua* inoculums (~10^8 CFU/ ml).

2.5.2 | Sample inoculation and treatments

Apple cubes were inoculated with *E. coli* and *L. innocua* before coating treatments to study the survival and growth of pathogenic bacteria. To carry this out, 1 ml of each inoculum previously prepared was applied by spray on apple to obtain a concentration of approximately 10^5 CFU per g of fruit. The inoculated apple was dried in a biosafety chamber with flowing air for 30–35 min and then coated (Rojas-Graü et al., 2007). Treatments applied were: gellan-based-solution enriched with apple fiber (GF) as control without biopreservatives, gellan-based-solution enriched with apple fiber plus geraniol (GF + ge) and gellan-based-solution enriched with apple fiber plus vanillin (GF + va). Coating and packaging procedures were carried out as was previously described

for the first study. Containers were stored at 5 °C and microbial counts were determined along the storage to find out the antimicrobial effect of geraniol and vanillin against pathogens. Non-inoculated apple cubes were also subjected to microbiological analyses during storage to investigate the presence or absence of *E. coli* and *Listeria* spp.

2.5.3 | Microbial analyses

Samples of 10 g of fruit were separated from each container. Homogenates and dilutions were prepared according to the method described in Section 2.4.1. Eosin methylene blue (EMB) agar (Britania, Argentina) was used for *E. coli* counts and plates were incubated for 24 hr at 37 °C Also, a chromogenic test kit (Chromobrit, Britania, Argentina) was used to confirm *E. coli* colonies (Alvarez, Ponce, Mazzucotelli, & Moreira, 2015). Oxford agar combined with Oxford selective supplement BS003 (Biokar Diagnostics, France) was employed for *Listeria* spp. counts. Plates were incubated during 24–48 hr at 35–37 °C (Cassani, Tomadoni, Ponce, Agüero, & Moreira, 2017). Results were recordered as log CFU/g. Microbial determinations were performed by duplicate at Days 0, 4, 8, and 12 using two containers per treatment and sampling time.

2.6 | Statistical analysis

A completely randomized design with two factors (coating treatment and time of storage) was used for each study. Results were shown as mean values followed by standard deviations. Statistical analyses were performed using InfoStat software (v2015) (Universidad de Córdoba, Córdoba, Argentina). Analysis of variance ANOVA ($p < .05$) was carried out and differences between treatments and through storage time were determined using the Tukey–Kramer multiple comparison test with a 95% confidence level. Assays were performed in triplicate on two independent experimental runs. Modeling of microbial growth curves was performed employing DMFit, a Microsoft Excel complementary tool (D-model, J. Baranyi, Institute of Food Research, Norwich, UK).

3 | RESULTS AND DISCUSSION

3.1 | Evolution of quality attributes along storage of apple cubes coated with gellan enriched with prebiotic fiber and biopreservatives

3.1.1 | Microbiological quality

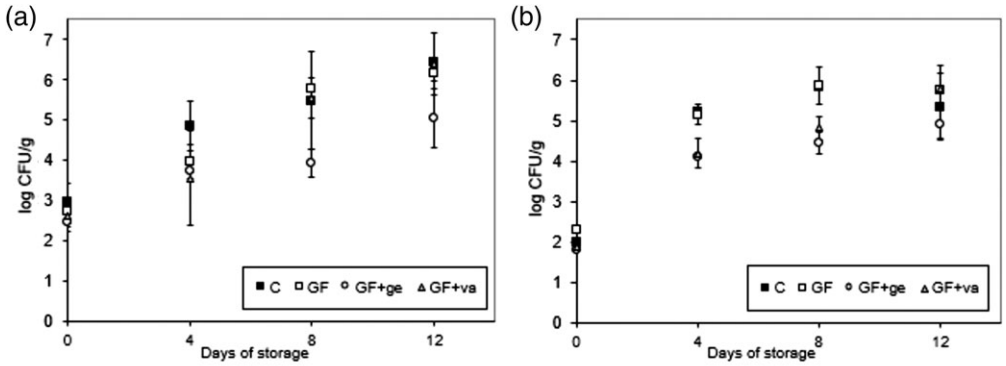
Figure 1a shows the evolution of mesophilic aerobic bacteria (MES) counts on treated and untreated apple cubes coated with gellan during refrigerated storage. At Day 0, MES counts of apples treated with vanillin and geraniol did not exhibit significant differences when compared to C and GF samples (ranged from 2.5 to 2.9 CFU/g). However, differences ($p < .05$) between MES counts on gellan-coated (with and without biopreservatives) and C apple cubes were observed at Day 4 of storage. Particularly, geraniol significantly inhibited MES growth when it was incorporated into gellan-based-coatings (GF + ge) showing a maximum growth rate significantly lower compared to C and GF treatment ($p < .05$; Figure 1a). MES counts on GF + ge samples stored during 12 days resulted lower than MES showed by the rest of samples (1.5 log reductions). It should be noticed that uncoated and gellan-fiber coated apple cubes (with and without biopreservatives) showed MES counts below the maximum limit (10^7 CFU/g) allowed in minimally processed foods according to the Spanish Regulation along the whole period of storage. Therefore, uncoated and coated samples continued to be safe for consumption after 12 days of cold storage and shelf life was not limited by microbial counts.

The development of yeast and molds (YM) population of coated and uncoated apples through storage is shown in Figure 1b. Initial YM loads ranged 1.8 and 2.3 log, without significant reducing effect by treatments immediately after dipping ($p > .05$). Increments observed in YM counts were in a range of 2.5–3.5 log units throughout 12 days of storage. Also, gellan-based coatings plus vanillin and geraniol significantly inhibited YM growth compared to C and GF samples. Lower values of growth rate were observed for these antimicrobial treatments than those obtained for controls of uncoated apple cubes (C) and gellan-coated apples without biopreservatives (GF). Moreover, YM maximum counts in GF + ge samples resulted significantly ($p < .05$) lower than those observed for C and GF samples (0.9–1.1 log reductions) (Figure 1b).

Recently, innovative edible coating formulations were assessed with the aim of reducing microbial growth in fresh cut apple. Thus, Pilon et al. (2015) successfully applied 110 nm chitosan nanoparticles on apple slices for controlling the growth of yeast and molds, mesophilic and psychrotrophic bacteria during 10 days of refrigerated storage. Moreover, Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, and Martín-Belloso (2015) used lemongrass oil nanoemulsions at 0.5 and 1% v/v which completely inhibited the proliferation of native microflora in apple pieces along two weeks of storage.

In accordance with our findings, Raybaudi-Massilia, Mosqueda-Melgar, and Martin-Belloso (2006) have demonstrated the effectiveness of geraniol as antimicrobial agent on ready-to-eat cut melon. It was added at 5 g/L in edible alginate-based coatings and prolonged the shelf life of fruits for 21 days at cold storage. Also, Cassani, Tomadoni, Viacava, Ponce, and Moreira (2016) reported that the incorporation of vanillin (1.8 g/L) and geraniol (0.4 ml/L) greatly reduced microbial load (reductions from 4 to 6 logs) of fiber-enriched strawberry juices stored for two weeks at 5 °C, compared to untreated juice. Moreover, Rupasinghe, Boulter-Bitzer, Ahn, and Odumeru (2006) working with different apple cultivars showed that the use of vanillin (1.8 g/L) applied within an antibrowning solution reduced mesophilic aerobic growth in apple wedges under refrigerated storage.

Vanillin exerted antimicrobial activity by altering membrane functions, dissipating ion gradients and by inhibiting bacterial respiration processes (Fitzgerald et al., 2004). Also, Fitzgerald, Stratford, Gasson, and Narbad (2005) proposed that the aldehyde moiety of this molecule is largely responsible for its antifungal capacity. Moreover, phenolic structure has a critical function in the antimicrobial activity of plant volatile oils, such as geraniol. This activity implicates loss of integrity of cell membrane, electron transport inhibition, protein translocation, and phosphorylation (Dorman & Deans, 2000).



	Treatment	Growth kinetic parameters † (mean value ± SE) Initial count log (CFU/g)	Maximum rate log (CFU/g)/ day	Maximum count log (CFU/g)	R^2
(A) Mesophilic bacteria	Control	3.18 ± 0.46 a	0.32 ± 0.07 a	6.49 ± 0.66 a	0,88
	GF	2.63 ± 0.25 ab	0.39 ± 0.06 a	6.20 ± 0.29 a	0,97
	GF+ge	2.62 ± 0.27 ab	0.20 ± 0.04 b	ND	0,91
	GF+va	2.50 ± 0.16 b	0.32 ± 0.02 a	ND	0,99
(B) Yeast and molds	Control	2.00 ± 0.34 a	0.84 ± 0.16 a	5.60 ± 0.24 a	0,96
	GF	2.30 ± 0.25 a	0.73 ± 0.03 a	5.80 ± 0.06 a	0,95
	GF+ge	1.80 ± 0.21 a	0.59 ± 0.08 b	4.69 ± 0.15 b	0,95
	GF+va	2.16 ± 0.33 a	0.56 ± 0.12 b	5.32 ± 0.24 a	0,91

† Baranyi (no lag phase) model. Different letters within columns indicate significant differences ($P < 0.05$).

FIGURE 1 Evolution and growth kinetics parameters of naturally occurring microbiota of fresh-cut apples coated with gellan-apple fiber plus biopreservatives during storage at 5 °C: (a) mesophilic bacteria; (b) yeasts and molds. Results are the mean of two independent experiments counted in duplicate. Bars indicate *SD*. C = Uncoated control; GF = Gellan-fiber; GF + ge = Gellan-fiber plus geraniol; GF + va = Gellan-fiber plus vanillin

3.1.2 | Antioxidant properties

The impact of gellan-fiber edible coatings with and without biopreservatives and refrigerated storage on antioxidant properties of fresh-cut apple is presented in Table 1 A significant correlation between TPC and antioxidant capacity of fresh-cut apples (untreated and treated) was found with a Pearson coefficient of 0.8.

Regarding total phenolic content of apples, initial value on uncoated apple cubes (C) was 106.3 mg GAE/ 100 g and differences between GF coated and C samples were not significant. Thus, apple fiber incorporated into the gellan solution did not provide a significant additional amount of phenolic compounds to the product, although this ingredient was obtained from a fruit by-product rich in phytochemicals. TPC of samples treated with gellan-fiber coating containing vanillin (GF + va) and geraniol (GF + ge) were significantly ($p < .05$) higher (23 and 19%, respectively) than their respective fiber-enriched control GF immediately after treatments application (Table 1). These increments were expected given that both antimicrobials are phenolic compounds itself. Although a sharp decrease in TPC was found along the first four days regardless the applied treatment, GF+ va showed the highest TPC values. From Days 8 to 12 of storage, neither vanillin nor geraniol showed improvements on TPC of apple cubes. In line with our findings, Tomadoni, Viacava, Cassani, Moreira, and Ponce (2016) observed an initial increase in polyphenol content of strawberry juice when vanillin was added at 2.5 and 5 g/L. However, TPC levels in treated and untreated juices remained with minimal changes along refrigerated storage period.

Antioxidant capacity of fresh apple cubes is shown in Table 1 as percent inhibition of DPPH radical. An initial DPPH inhibition of 65%

TABLE 1 Effect of gellan-biopreservatives edible coatings enriched with apple fiber on antioxidant properties of fresh-cut apples stored at 5 °C for 12 days

		Storage time (days)							
Parameter	**Treatment**	**0**		**4**		**8**		**12**	
Total phenolic content (mg GAE/100 g)	C	106.3 ± 9.8[a]	Ba	81.4 ± 2.1	Bb	74.8 ± 0.6	Bb	81.5 ± 8.3	Bb
	GF	98.8 ± 2.0	Ba	83.1 ± 6.6	Bc	92.7 ± 5.8	Ab	94.3 ± 7.1	Aab
	GF + ge	130.9 ± 5.8	Aa	81.2 ± 10.7	Bc	87.4 ± 4.1	Ab	82.0 ± 4.0	Bbc
	GF + va	126.3 ± 10.2	Aa	93.5 ± 15.7	Ab	92.9 ± 10.7	Ab	85.5 ± 2.3	Bb
DPPH inhibition %	C	65.1 ± 8.1	BCa	44.2 ± 2.2	Ac	49.8 ± 3.0	Bb	40.1 ± 2.8	ABd
	GF	60.8 ± 1.2	Ca	38.0 ± 2.8	Bc	60.2 ± 12.0	Aa	43.2 ± 4.4	Ab
	GF + ge	73.2 ± 0.6	Aa	42.0 ± 3.1	ABc	48.0 ± 2.3	Bb	41.8 ± 1.5	ABc
	GF + va	66.4 ± 6.8	Ba	44.3 ± 7.6	Ab	45.6 ± 3.1	Bb	39.9 ± 0.5	Bc

C = uncoated control; GF = gellan-fiber; GF + ge = gellan-fiber plus geraniol; GF + va = gellan-fiber plus vanillin.

[a] Data is shown as means ± *SD*. Values in the same column with different capital letter indicate significant differences ($p < .05$) between treatments. Values in the same row for each treatment with different lowercase letter indicate significant differences ($p < .05$) through storage time.

was observed for uncoated fresh-cut apples (C). The addition gellan with apple fiber did not exert changes in the initial antioxidant capacity of apple cubes. Moreover, only the incorporation of geraniol caused a significant increment ($p < .05$) in antioxidant activity of coated apples just after processing, compared to C and GF treatment. Finally, the antioxidant capacity values of treated and untreated apple cubes showed a similar evolution throughout storage time without significant effects of the bioactive compounds used. Tomadoni et al. (2016) assessed the impact of bioactive compounds such as geraniol and vanillin on the antioxidant activity of strawberry juice measured by DPPH method. In agreement with our findings, these authors concluded that none of the bioactive agents increased the antioxidant activity of the product in comparison to untreated samples during cold storage.

3.1.3 | Color parameters

The impact of gellan-fiber edible coatings, with or without the use of biopreservatives, on L^* and h° parameters of apple cubes stored at 5 °C is shown in Table 2. Color parameters evolution during storage time was similar for all samples. Therefore, mean values representing the whole storage period are shown in Table 2. L^* and h° values tended to decrease in all samples regardless of the applied treatment, mainly during the first four days of storage. Decreasing h° values indicate a gradual darkening of apple cubes. Regarding treatment effects, it was observed that GF coating application significantly reduced L^* ($p < .05$), compared to C, on average throughout storage time. Changes in the surface reflection properties that occur when fruits are coated may explain this decreasing effect on luminosity. Finally, the use of gellan-fiber coating plus vanillin exerted a significant positive effect ($p < .05$) on color by preventing the decrease of L^* and h° in fresh-cut apples. At the same time, this sample presented lower browning (sensory evaluation) and higher firmness values during the whole storage period, compared to C samples. In contrast, apple cubes treated with GF coating and GF plus geraniol presented lower h° values with respect to control sample C on average throughout storage period. The addition of geraniol (GF + ge) to the coating did not introduce changes in L^* and h° parameters ($p > .05$) compared to GF, meanwhile these values were significantly lower ($p < .05$) than those obtained for C.

The antibrowning effect of ascorbic acid and its derivatives (at 0.5–4%) has been widely demonstrated in several fresh-cut fruits including apples (Soliva-Fortuny & Martín-Belloso, 2003). For this reason, ascorbic acid dipping solution was used as a common treatment to delay browning in all samples including uncoated control. Coatings act as oxygen barriers that may reduce the surface modifications associated with polyphenol oxidase (PPO) activity in fresh-cut-fruits and thus, minimizing color changes (Zambrano-Zaragoza, Mercado-Silva, Gutiérrez-Cortez, Cornejo-Villegas, & Quintanar-Guerrero, 2014). However, the GF coatings used in our study did not improve color attributes compared to uncoated sample probably due to the antibrowning action of ascorbic acid applied in all samples. In accordance with our results, Moreira, Alvarez, Martín-Belloso, and Soliva-Fortuny (2017) reported that pectin-coated apple cubes did not show any difference in color attributes with respect to an uncoated sample when both were dipped in ascorbic acid solution.

Edible coatings have been developed in an effort to delay color changes due to browning reactions in Red Delicious apple pieces. In this respect, Zambrano-Zaragoza, Gutiérrez-Cortez, et al. (2014) formulated a coating using tocopherol/mucilage nanoemulsions with significant antibrowning properties which reduced PPO activity and browning index. Also, nano-coatings with α-tocopherol and xanthan gum showed high potential to preserve apple color reducing the rate of change in L^* and browning index (Zambrano-Zaragoza, Mercado-Silva, et al., 2014).

Regarding the use of vanillin, Rupasinghe et al. (2006) tested its impact on color attributes of apples wedges. Non-significant effects were found when this compound was incorporated to a commercial calcium ascorbate dipping solution at 12 mM.

3.1.4 | Firmness

Loss of firmness greatly affects visual quality of plant tissues along prolonged storage and it is due to metabolic activity and changes in

TABLE 2 Effect of gellan-biopreservatives edible coatings enriched with apple fiber on color parameters (lightness L^*, hue angle h°) and firmness of fresh-cut apples stored at 5 °C for 12 days

		Storage time (days)					
Parameter	Treatment	0	4	8	12	Mean	
L^*	C	79.74 ± 0.94[a]	77.83 ± 2.08	76.74 ± 2.18	78.94 ± 2.12	*78.31*	a
	GF	80.19 ± 2.02	73.98 ± 3.79	74.08 ± 4.67	72.61 ± 5.48	*75.22*	b
	GF + ge	78.14 ± 1.29	74.92 ± 6.11	75.79 ± 2.35	73.29 ± 4.22	*75.33*	b
	GF + va	80.22 ± 1.37	78.06 ± 1.65	78.28 ± 2.25	75.85 ± 3.20	*78.10*	a
h°	C	82.63 ± 0.75	77.51 ± 0.95	77.03 ± 1.24	78.57 ± 2.12	78.94	b
	GF	81.96 ± 0.60	77.68 ± 1.68	76.14 ± 1.48	76.63 ± 1.27	*78.10*	c
	GF + ge	81.68 ± 1.79	77.39 ± 1.77	76.81 ± 1.47	76.06 ± 1.61	*77.99*	c
	GF + va	83.38 ± 0.57	80.04 ± 1.07	79.57 ± 1.83	79.78 ± 1.11	*80.69*	a
Firmness (N/cm^2)	C	59.8 ± 5.8	63.2 ± 8.0	70.4 ± 5.5	68.6 ± 3.1	*65.5*	b
	GF	66.0 ± 7.5	62.1 ± 7.4	71.2 ± 5.9	70.7 ± 9.3	*67.5*	ab
	GF + ge	56.6 ± 4.3	61.8 ± 10.8	72.1 ± 3.4	66.8 ± 4.1	*64.3*	b
	GF + va	62.0 ± 7.9	71.9 ± 3.3	73.8 ± 3.6	75.8 ± 3.6	*70.2*	a

C = uncoated control; GF = gellan-fiber; GF + ge = gellan-fiber plus geraniol; GF + va = gellan-fiber plus vanillin.
[a] Data is shown as means ± *SD* ($n = 20$). Mean values (representative of all sampling times) with different letters indicate significant differences ($p < .05$).

water content (Rojas-Graü, Tapia, & Martín-Belloso, 2008). Firmness values of treated and untreated apple cubes stored up to 12 days are listed in Table 2. Firmness evolution during storage was similar for all samples; for that, mean values representing the whole storage period are shown in Table 2. Uncoated and coated samples were able to maintain or even improve their firmness along storage. As it was described in the methodology section, both coated and non-coated apple cubes were subjected to the same calcium chloride treatment. GF coating did not significantly ($p > .05$) affect firmness of apple cubes compared to uncoated C. The action of calcium chloride as an additive to maintain firmness has been widely studied. According to King and Bolin (1989), this agent combines with pectic acid leading to the formation of calcium pectate in the cell wall and consequently, strengthening molecular bonds between cell wall components. Lee, Park, Lee, and Choi (2003) found that the use of calcium chloride at 10 g/L in coated fresh-cut apples was useful to retain fruit firmness. Also, Moreira et al. (2015) obtained similar results working with gellan coated apple cubes followed by an immersion in a 20 g/L calcium chloride solution.

In our study, the coating treatment did not show improvement on firmness when compared to an uncoated sample also treated with calcium chloride. Other studies showed firmness enhancement by the use of edible coating using as control samples apples dipped in distilled water. In this way, Espino-Díaz, Molina-Corral, Sepulveda, González-Aguilar, and Olivas (2016) observed higher firmness retention in apple wedges by the use of alginate-based formulations. Similar findings were reported by Saba and Sogvar (2016) working with fresh-cut-apple coated with carboxymethyl cellulose solutions.

Regarding the influence of preservatives, the enrichment of gellan-fiber coating with vanillin significantly affected ($p < .05$) the fruit firmness. GF + va coated cubes exhibited the highest firmness measurements during cold storage (Table 2). The capacity of vanillin as an inhibitor of microbial growth, especially of yeasts and molds, was previously described in our report. Thus, vanillin contributes to firmness maintenance preventing texture damage caused by the metabolic activity of these microorganisms. In accordance with our results, Rojas-Graü et al. (2007) established that vanillin (3 and 6 g/kg) added to alginate coatings maintained fresh-cut apple firmness while the use of oregano and lemongrass essential oils had deleterious effects on fruit firmness. Contrarily, GF + ge coated cubes presented lower values of firmness even than uncoated sample (C). This effect of geraniol on texture was previously reported by other authors. Tomadoni, Moreira, Pereda, and Ponce (2018) obtained several losses of firmness from the seventh day of storage when assessed fresh-cut strawberries coated with gellan added with geraniol. Also, Raybaudi-Massilia, Mosqueda-Melgar, and Martín-Belloso (2008) observed a decrease in this attribute on fresh-cut melon when incorporated essential oils at high concentrations into alginate coatings. These authors affirmed that geraniol affected firmness in higher proportion than other active compounds added. The negative effect of geraniol on fruit firmness could be attributed to structural changes on cellular tissues affecting directly the firmness, or promoting the release of enzymes and substrates helped by cutting operations (Raybaudi-Massilia et al., 2008; Tomadoni et al., 2018).

3.1.5 | Sensory evaluation

Figure 2 displays the overall visual quality (OVQ), color, odor, and flavor scores for apple cubes treated with gellan-apple fiber coatings with and without biopreservatives. Initially (Day 0), fresh-cut apples enriched with apple fiber incorporated into the gellan coating (GF) could not be discriminated from fresh-cut control sample (C); no significant differences ($p > .05$) in sensory attributes between these samples were observed. Also, the taste for all samples was acceptable. At Day 4, it was noticed that cut fruit treated with gellan functional coating treated with both biopreservatives (GF + ge and GF + va) obtained significantly ($p < .05$) higher OVQ (3.4 and 3.9, respectively) and color (3.6 and 3.8, respectively) scores compared to control C and GF samples (2.8 and 1.7 for OVQ, 2.6 and 1.7 for color, respectively). Conversely, the corresponding odor scores were significantly lower ($p < .05$) than C and GF controls indicating that strange odors were detected as consequence of the use of these volatile compounds, although acceptability of samples was not compromised. From Days 8 to 12 of storage, GF + va apple cubes obtained the highest scores ($p < .05$) for OVQ and color compared to the rest of samples. The characteristic odor imparted by vanillin continued to be perceived. In contrast, scores obtained for C, GF, and GF + ge samples fell below the limit of acceptability (2.5) at Day 8. Therefore, extended storage periods (12 days) for fresh-cut apple can be achieved through the application of GF + va functional coating with scores above the limit (2.5) for all sensory attributes evaluated. From Day 8 and until the end of storage, flavor was not evaluated in apple samples due to safety reasons according to the microbiological limit established by Spanish Regulation.

3.2 | Pathogen survival: Impact of coatings

The evolution of *E. coli* O157:H7 and *L. innocua* populations in inoculated apple cubes along storage is shown in Figure 3a,b. Absence of endogenous *Listeria* spp. and *E. coli* in non-inoculated apple samples was confirmed at each sampling time. Results showed that inoculated *E. coli* population did not increase but survived in apple cubes for 12 days under refrigerated conditions. Similar results were found by Abadias, Alegre, Oliveira, Altisent, and Viñas (2012) working with *E. coli* O157:H7 inoculated on minimally processed fruits and vegetables and maintained at 5 °C. Regarding the effect of treatments, it was observed that geraniol and vanillin exerted an initial bactericidal effect against *E. coli*, reducing counts in approximately 0.7–0.8 log CFU/g. Moreover, geraniol showed a significant bacteriostatic effect from 8th day (1.4–1.7 log lower than GF coated samples; Figure 3a).

L. innocua was able to grow in gellan-fiber coated apples for the first four days of storage. During this period, gellan-fiber coating plus both biopreservatives significantly inhibited the growth of *L. innocua* achieving count reductions of 1.9 log. From Days 8 to 12 of storage, this inhibitory effect disappeared (Figure 3b). Up to the end of storage, GF samples (control without biopreservatives) showed counts of *E. coli* and *L. innocua* similar to initial pathogen loads at Day 0 (Figure 3a,b). Several factors may explain this effect, including the low storage temperature, low pH of substrate, and the presence of competitive microflora.

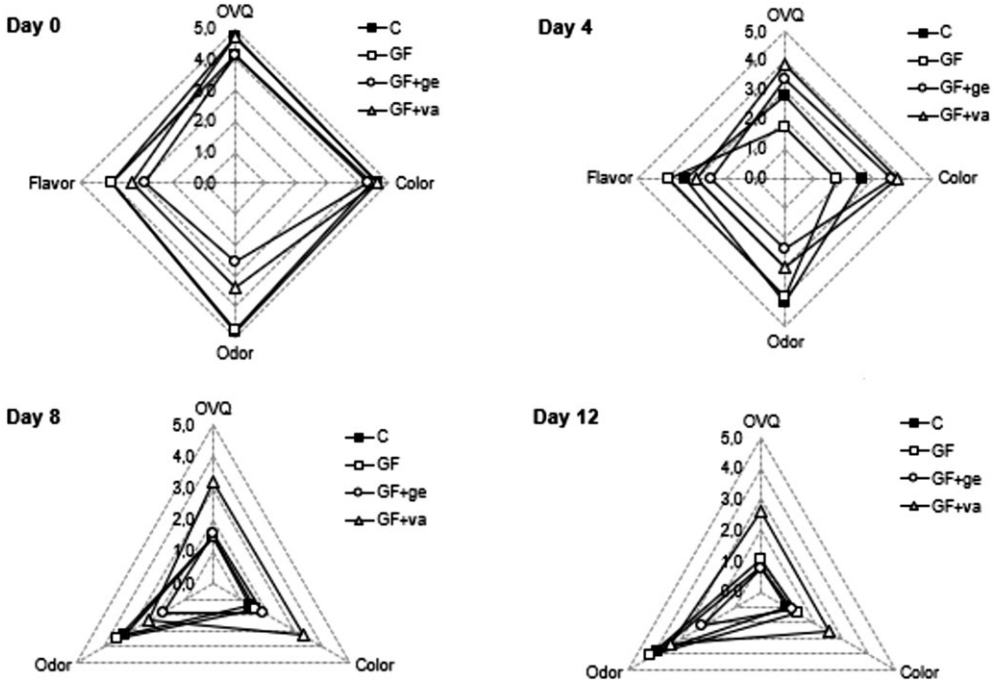


FIGURE 2 Changes in sensory attributes of fresh-cut apples: Effect of gellan-biopreservatives edible coatings (enriched with apple fiber) and refrigerated storage time. C = Uncoated control; GF = Gellan-fiber; GF + ge = Gellan-fiber plus geraniol; GF + va = Gellan-fiber plus vanillin

The antimicrobial activity of vanillin was previously studied by Rupasinghe et al. (2006) using pathogen and spoilage microorganisms frequently associated with fresh-cut apples such as *E. coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. *enterica* serovar Newport, *Penicillium expansum*, *Saccharomyces cerevisiae*, and others. These authors found MIC values ranging 0.9–2.7 g/L being *P. expansum* the most resistant with MIC >2.7 g/L. Vanillin concentration used to formulate the film-forming solution applied in our study is 2.5 g/L (Tomadoni et al., 2015), similar to the highest value of that MICs range. Regarding geraniol antimicrobial activity, Tomadoni et al. (2015) found 0.6 and 0.5 ml/L as MIC values when pure cultures of *E. coli* O157:H7 and *L. monocyogenes*, respectively, were tested. Moreover, Scortichini and Rossi (1991) tested geraniol as antimicrobial against seven strains of *Erwinia amylovora* and found that this compound inhibited the growth of all microorganisms at concentrations ranging 0.6–1.5 ml/L.

Some in vivo studies about the effectiveness of vanillin and geraniol for controlling *E. coli* and *Listeria* on fruit-based juices and minimally processed fruits have been developed. For instance, Rojas-Graü et al. (2007) studied the effect of vanillin and other essential oils added into alginate-apple puree edible coatings on the survival of *L. innocua* in Fuji apple pieces. When vanillin was incorporated into coatings at 3 and 6 g/kg, significant reductions in *L. innocua* counts were observed (3 log units) during the first week of storage compared to the initial inoculum load. This effect remained until 21 days of storage. In this case, the applied vanillin concentrations were higher in comparison with those used in our work.

Our results are in agreement with Raybaudi-Massilia et al. (2006) who found reductions on *E. coli*, *L. innocua*, and *Salmonella Enteritidis*

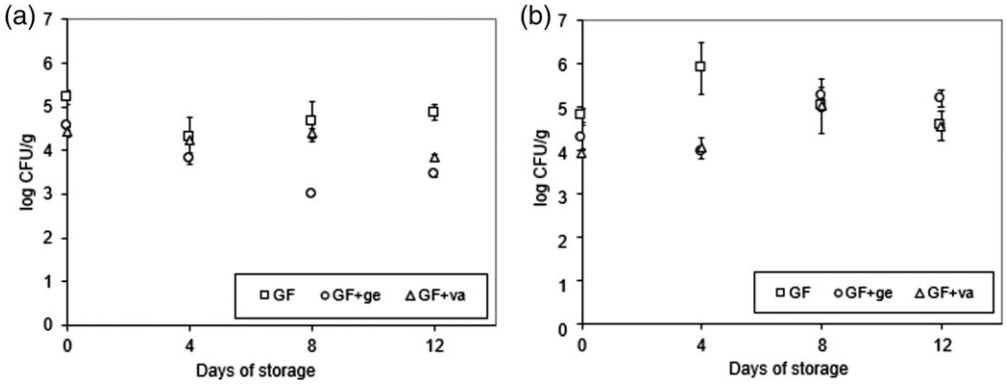


FIGURE 3 *E. coli* O157:H7 (a) and *L. innocua* (b) survival in inoculated fresh-cut apples, coated with gellan-apple fiber plus biopreservatives during storage at 5 °C. Results are the mean of two independent experiments counted in duplicate. Bars indicate *SD*. GF= Gellan-fiber; GF + ge = Gellan-fiber plus geraniol; GF + va = Gellan-fiber plus vanillin

inoculated in apple juices subjected to geraniol treatments (2 ml/L). Furthermore, Tomadoni et al. (2016) evaluated the impact of different doses of vanillin and geraniol on *E. coli* O157:H7 survival on inoculated strawberry juice. It was reported that vanillin at 5 g/L and geraniol at 0.6 ml/L were capable to reduce initial *E. coli* counts (2 logs) in comparison to untreated juice. Also, Cassani et al. (2017) demonstrated that the use of vanillin (1.25 g/L) combined with ultrasound treatment (7.5 min at 40 kHz) on strawberry juice successfully reduced inoculated *E. coli* O157:H7 and *L. innocua* counts to non-detectable values after 7 days of refrigerated storage. In our work, a lower effectiveness of both antimicrobial compounds was observed probably due to differences in plant substrates, different application methods (edible coatings compared to direct application on juice) and less contact pathogen-compound.

4 | CONCLUSION

Gellan functional coatings developed by combining prebiotic fiber and natural preservatives showed different advantages regarding quality and safety of ready-to-eat apples cubes. The use of gellan-fiber edible coating plus geraniol retarded the microbial spoilage of apples and, also, successfully reduced *E. coli* and *L. innocua* populations on inoculated fresh-cut apple through storage period. Conversely, gellan-fiber coating plus vanillin showed beneficial results: they inhibited yeasts and molds growth, enhanced firmness retention of apple cubes, and better maintained their typical color attributes during the entire storage period. Moreover, the enrichment of gellan coating with apple fiber together with the addition of vanillin allowed the improvement of OVQ, color, and sensory acceptability of fresh-cut apples, extending the shelf-life at least 4 days compared with other samples. Both bioactive compounds combined with apple fiber and added into gellan coatings might be good alternatives for enhancing quality attributes and assuring safety of ready-to-eat apples. It is important to conclude that vanillin showed the greatest potential to be used technologically to produce a commercial fruit product given that significant improvements in the overall quality of apple cubes were achieved.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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