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Gellan-based coatings incorporated with natural antimicrobials in fresh-cut strawberries: Microbiological and sensory evaluation through refrigerated storage

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ABSTRACT

Gellan (Gel) coatings (0.5% w/v) were tested on quality parameters of fresh-cut strawberries through storage. Geraniol (G) and Pomegranate extract (PE) were incorporated at different concentrations according to their solubility and stability on the film forming solutions: 1.2 and 2.4 μL/mL of G (Gel + G1 and Gel + G2, respectively), and 360 and 720 μg/mL of PE (Gel + PE1 and Gel + PE2, respectively). Fresh-cut strawberries were immersed in the different solutions and were stored (7 days, 5 °C) in order to assess the evolution of mesophilic bacteria (MES), yeast and molds (YM) and psychrophilic bacteria (PSY). Furthermore, at days 0 and 7, a sensory evaluation was performed. Gel coatings (without bioactive compounds) had no significant effect on native microflora compared to control. Gel + G coatings at both concentrations tested significantly reduced microbial counts, compared to untreated sample, improving the microbiological stability with respect to control. On the other hand, the incorporation of PE was not able to control the microbial growth of the product. With regards to sensory quality, the texture of the fresh-cut strawberries was significantly affected by storage time, and the tested treatments were not able to improve this attribute. In fact, samples with coatings incorporated with geraniol showed a bigger firmness loss than control.

1. Introduction

Minimally processed (MP) foods aroused as an alternative for consumers who demand convenient products, with sensory characteristics similar to those of the fresh produce. However, due to operations such as peel and cut, MP fruits and vegetables are highly perishable, susceptible to physical, enzymatic and microbiological deterioration, negatively damaging the product's organoleptic quality and the consumers' health.

Recently, different technologies are being investigated to preserve MP fruits and vegetables. Among these, the use of edible coatings (EC) is a promising alternative to extend the shelf-life of MP products (Alvarez, Ponce, & Moreira, 2013). EC form a semipermeable barrier against gases and vapor, and can maintain or even improve the quality, safety and stability of coated food products. Different edible coatings have been applied to foods with good results in terms of quality parameters (Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan, 2013; Rossi Marquez et al., 2017; Sanchís et al., 2016).

The most common edible coatings in the food industry are those with matrices based on polysaccharides and proteins, such as gellan gum. Gellan gum (Gel), is formed by a polysaccharide of high molecular weight. This polysaccharide of microbial origin is produced by the carbohydrate fermentation in pure culture of *Sphingomonas elodea* (formerly known as *Pseudomonas elodea*). The repetitive unit of this polysaccharide consists of the tetrasaccharide formed by two residues of D-glucose, linked to residues of L-rhamnose and D-glucuronic acid (Azarakhsh et al., 2013). These tetrasaccharide units are linked together by α (1–3) glycosidic bonds to form the polysaccharide in question. Gellan gum is soluble in water and insoluble in ethanol, and it has unique colloidal and gelling properties, which provides good capacity to form films and coatings (Bajaj, Survase, Saudagar, & Singhal, 2007).

Furthermore, EC are usually used as carriers of different food additives, such as, antibrowning agents, antioxidants, antimicrobials, colorants and flavoring agents (Alvarez, Ponce, Moreira, & del, 2013; Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015; Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008; Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009; Rojas-Graü, Tapia, Rodríguez, Carmona, & Martin-

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Belloso, 2007). Different studies have been reported where antimicrobial agents have been applied to a variety of EC to extend the shelf-life of fresh-cut fruits. For example, Brasil, Gomes, Puerta-Gomez, Castell-Perez, and Moreira (2012) evaluated the incorporation of cinnamaldehyde into chitosan and pectin coatings on papaya; Salinas-Roca, Soliva-Fortuny, Welti-Chanes, and Mart?n-Belloso (2016) evaluated the incorporation of mallic acid into alginate coatings on mango, combined with light pulses. Besides, the incorporation of essential oils into alginate EC reduced the microbial growth in fresh-cut pineapple (Nima Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan, 2014) and in fresh-cut apples (Rojas-Graü et al., 2007).

Among natural compounds, geraniol, a monoterpene alcohol ($C_{10}H_{18}O$), has potential application in the food industry. It is a common constituent of a wide variety of essential oils (such as, geranium, citronella, lemon and roses), produced by flowers of different species and present in the vegetal tissues of many herbs (Chen & Viljoen, 2010). There is strong evidence with respect to the antioxidant, antimicrobial and antitumoral activity of geraniol (Ahmad et al., 2011; Kim et al., 2011; Madankumar et al., 2013; Nazer, Kobilinsky, Tholozan, & Dubois-Brissonnet, 2005; Tiwari & Kakkar, 2009; Yegin, Perez-Lewis, Zhang, Akbulut, & Taylor, 2016).

Another natural agent with potential as food biopreservative is pomegranate extract obtained from *Punica granatum* L. There are several studies that have demonstrated the antimicrobial efficacy of various pomegranate extracts and also of the pure compounds obtained from different parts of the plant. The effectiveness of these extracts to inhibit spoilage and pathogen bacteria growth has been demonstrated, besides of presenting antifungic and antiviral activity (Dahham, Ali, Tabassum, & Khan, 2010; Howell & Souza, 2013; Pagliarulo et al., 2016; Su, Sangster, & D'Souza, 2010). The phytochemicals present in the pomegranate extract, such as phenolic compounds, tannins, flavonoids and alkaloids, are responsible for its biological properties. Hence, the use of pomegranate extract or its pure compounds as food biopreservatives is promising. The principal bioactive compounds present in pomegranate extracts are punicalagins, ellagic acid and its derivatives (Qu et al., 2012).

Antimicrobial activity of these natural compounds has been demonstrated in the past through *in vitro* studies (Alvarez, Moreira, & Ponce, 2012; Fitzgerald et al., 2004; Raybaudi-Massilia & Mosqueda-Melgar, 2006; Tomadoni, Cassani, Moreira, & Ponce, 2015; Vasantha Rupasinghe, Boulter-Bitzer, Ahn, & Odumeru, 2006). However, few studies have been made where these natural agents are applied *in vivo* in fresh-cut fruits. Hence, the main objective of this study was to evaluate the application of gellan edible coatings incorporated with geraniol or pomegranate extract on the microbial and sensory quality of fresh-cut strawberry through refrigerated storage.

2. Materials and methods

2.1. Materials

Gellan gum (Food grade, Kelcogel®) was purchased from CPKelco (Chicago IL, USA). For the films incorporated with bioactives, geraniol (purity ≥ 97% GC) was purchased from Sigma Aldrich (St Lois MO, USA), and pomegranate extract from PureBulk (USA). Characterization of the pomegranate extract used in the present study was determined by HPLC analysis, according to Tomadoni, Moreira, Espinosa, and Ponce (2017): ellagic acid (34.5%), gallic acid (18.4%), punicalagin A (9.8%), punicalagin B (4.7%), caffeic acid (1.9%). Glycerol was used as a plasticizer and was purchased from Biopack (Buenos Aires, Argentine).

2.2. Preparation of film forming solutions

Film forming solutions were prepared according to Moreíra et al. (2015), by dissolving gellan gum in distilled water (0.5% w/v) under magnetic stirring at 70 °C. Glycerol was incorporated as plasticizer at a concentration of 0.56% (w/v). This solution with no biopreservatives was used as a control (Gel edible coating).

Film solutions incorporated with bioactive compounds were prepared at different concentrations of geraniol and pomegranate extract according to previous assays, where the solubility and stability of each compound was tested in the gellan gum film forming solution. Hence, geraniol was incorporated into Gel films at two different concentrations: 1.2 and 2.4 μL/mL (Gel + G1 and Gel + G2 coatings, respectively). Geraniol was dispersed in the gellan solution with an Ultra-Turrax (Ika T25, USA) at 14.000 rpm for 15 min. Pomegranate extract was incorporated at 360 and 720 μg/mL (coatings Gel + EG1 and Gel + EG2, respectively) directly into the gellan solutions previously prepared.

2.3. Fruit processing, treatment application and storage conditions

Strawberries (*Fragaria* x *ananassa*) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentine. Fruits with uniform size and free of physical damage and fungal infection were selected. Strawberries were washed by immersion in tap water for 2 min, the stems were removed manually, and the fruits were cut in halves. The application of the treatments consisted of immersion of the cut strawberries in the different film forming solutions for 2 min, and the excess of coating solution was allowed to drip off.

Strawberry halves from each treatment (ca. 50 g) were placed into polypropylene sterile trays of dimensions 152.5 mm × 112.0 mm x 47.0 mm (Celpack S.A., Argentine). The trays were wrap-sealed in 25.4 μm thick polypropylene film (with permeability to O_2, CO_2 and water vapor of 3.08×10^{-4}, 2.05×10^{-3} and 2.05×10^{-6} $mmol^3 m^{-2} s^{-1}$ respectively, at P = 101325 Pa, T = 25 °C) using a manual thermo-sealing machine (HL, FS-300, Argentina). Finally, samples were stored at 5 °C in order to assess quality parameters. Two trays of each treatment were analyzed immediately after processing (day 0), and after 2, 5 and 7 days of refrigerated storage to evaluate the impact of gellan-based coatings on microbiological and sensory quality of minimally processed strawberries.

2.4. Microbiological analysis

Mesophilic bacteria (MES), yeast and molds (YM) and psychrophilic bacteria (PSY) were assessed to evaluate the impact of the different coatings on native microflora of MP strawberries through refrigerated storage. A portion of 10 g from each treatment, obtained randomly from different strawberry pieces, was aseptically removed from each tray and transferred into sterile plastic bags. Samples were diluted with 90 mL of peptonated water (0.1% w/v) and homogenized for 1 min in a stomacher blender. Serial dilutions (1:10) of each sample were made in sterile peptonated water (0.1% w/v) and surface spread by duplicate.

The enumeration of the microbial populations was performed according to Ponce, Agüero, Roura, Del Valle, and Moreira (2008) by using the following culture media and culture conditions: mesophilic aerobic bacteria on Plate Count Agar (PCA) incubated at 35 °C for 48 h; psychrophilic bacteria on PCA incubated at 7 °C for 7 d; yeast and molds on Yeast-Glucose-Chloramphenicol (YGC) medium incubated at 25 °C for 5 d. All culture media were purchased from Britania (Buenos Aires, Argentine). Colonies were counted and the results expressed as CFU/g of strawberries. Analyses were carried out periodically during 7 days in randomly sampled pairs of trays. Two replicate counts were performed for each tray.

2.5. Sensory analysis

A quantitative descriptive analysis (QDA) was performed according to Carbonell, Izquierdo, and Carbonell (2007). Ten trained panelists evaluated the fresh-cut strawberries prepared on the same day of the test (t = 0 d) and after 7 days of refrigerated storage at 5 °C. Samples

Table 1
Description of the selected sensory attributes, anchor ends and consensus values for the reference sample.

Attribute	Description	0	5	Ref.
Appearance				
OVQ	Overall visual quality	Poor	Excellent	5
Brightness	Intensity of brightness	Low	High	5
Odor				
Characteristic odor	Intensity of strawberry odor	Low	High	5
Off-odor	Intensity of fermentation or other non-characteristic odors	Low	High	0
Taste				
Sweetness	The intensity of sweet	Low	High	3
Acidity	The intensity of acid	Low	High	2
Texture				
Texture	Firmness	Very soft	Normal	5

Ref.: Reference: fresh-cut strawberry without coating.

labeled with 3 digit code numbers were randomly provided to the panelists.

Water was provided to panelists for eliminating the residual taste between samples. The attributes evaluated were: *overall visual quality* (OVQ), *characteristic odor*, *off-odor*, *sweetness*, *acidity* and *texture*. Unstructured line scales (5 cm) anchored at the ends with terms related with minimum and maximum intensities were used to evaluate each attribute. Definitions, anchor terms and reference values (fresh cut strawberry with no treatment) for each attribute are shown in Table 1.

2.6. Statistical analysis

A completely randomized design was used. Three independent runs were performed. Data obtained was analyzed using R v. 2.12.2. (R Development Core Team, 2011). Results reported in this article are mean values accompanied by their standard errors (Kuehl, 2001). Analysis of variance ANOVA was performed and Tukey-Kramer comparison test was used to estimate significant differences between treatments ($p < 0.05$) and between storage days ($p < 0.05$).

3. Results and discussion

3.1. Effect of gellan coatings on native microflora of fresh-cut strawberries

3.1.1. Mesophilic bacteria

Fig. 1 shows the evolution of mesophilic bacteria on fresh-cut strawberries treated with different gellan coatings. Initial MES count in control sample (without coating) was 4.81 log CFU/g, showing a significant increment throughout storage, reaching 7.40 log by day 7. Sample treated with Gel coating (without biopreservatives) showed similar counts to control during storage, as well as both treatments with pomegranate extract (Gel + PE1 and Gel + PE2). However, coatings incorporated with geraniol (Gel + G1 and Gel + G2) exerted a significant effect on MES counts, which did not increase significantly within the first 5 days of storage. MES growth was significantly reduced in Gel + G samples compared with control, with counts from 4.35 to 5.16 log in the entire period of storage.

3.1.2. Yeast and molds

Yeast and molds (YM) growth on fresh-cut strawberries treated with gellan coatings is shown in Fig. 2. The observed trend in the evolution of YM was similar to that above-described for MES. During storage, YM counts on control samples showed a significant growth, with initial values of 4.87 log, reaching 6.62 log CFU/g by day 7. Gel, Gel + PE1 and Gel + PE2 coatings showed no significant effect on YM counts with respect to control at day 0, as well as during the rest of the storage period. Gel + G1 coating did not decrease significantly the initial yeast and molds counts, but it did show a fungistatic effect, maintaining the

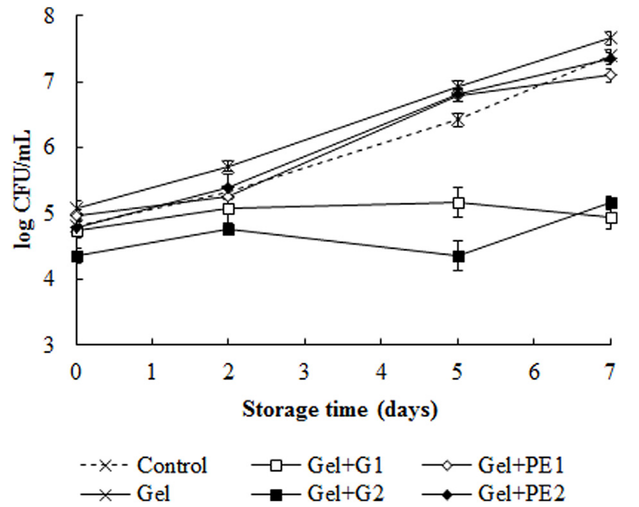

Fig. 1. Evolution of mesophilic bacteria in fresh-cut strawberries with gellan-based coatings throughout refrigerated storage at 5 °C. Bars indicate standard errors. Gel: gellan gum coating (0.5% w/v); G1: 1.2 µL/mL of geraniol; G2: 2.4 µL/mL of geraniol; PE1: 360 µg/mL of pomegranate extract; PE2: 720 µg/mL of pomegranate extract.

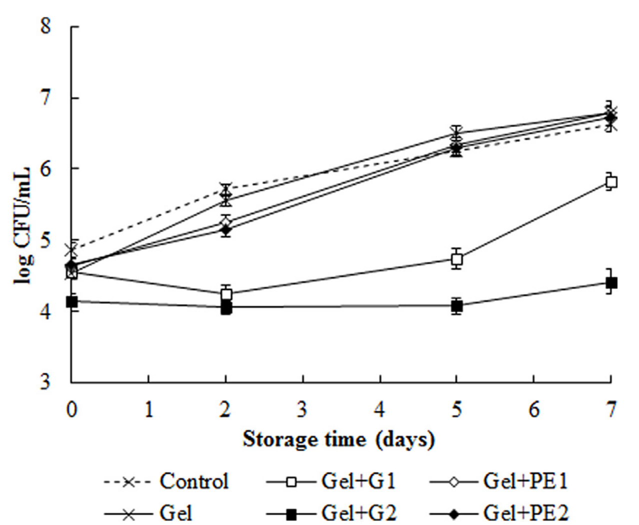

Fig. 2. Evolution of yeast and molds in fresh-cut strawberries with gellan-based coatings throughout refrigerated storage at 5 °C. Bars indicate standard errors. Gel: gellan gum coating (0.5% w/v); G1: 1.2 µL/mL of geraniol; G2: 2.4 µL/mL of geraniol; PE1: 360 µg/mL of pomegranate extract; PE2: 720 µg/mL of pomegranate extract.

counts at 4.74 log until day 5. At day 7, YM counts increased in Gel + G1 to 5.82 log; however, this value was significantly lower compared to the control sample. The effect of Gel + G2 was even greater, with an initial reduction of 0.71 log with respect to control. This reduction was significant from statistical point of view though it is not biologically significant. However, through the storage period, Gel + G2 exerted a fungistatic effect (with YM counts between 4.06 and 4.43 log CFU/g).

3.1.3. Psychrophilic bacteria

Fig. 3 shows the effect of the different gellan coatings on psychrophilic bacteria (PSY) of fresh-cut strawberries through refrigerated storage. Strawberries with no treatment showed a significant increment of PSY counts through time, similar to that observed in the other studied microbial populations, with an initial count of 4.38 log CFU/g and a final count of 7.37 log (day 7). Like YM and MES, Gel, Gel + PE1 and Gel + PE2 coatings did not show a significant effect in the reduction of

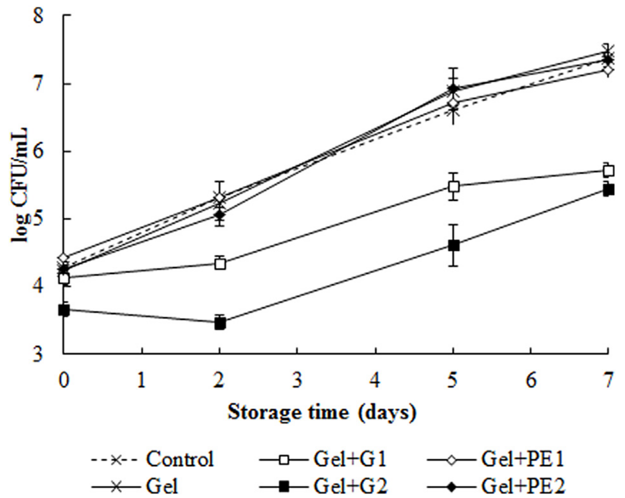

Fig. 3. Evolution of psychrophilic bacteria in fresh-cut strawberries with gellan-based coatings throughout refrigerated storage at 5 °C. Bars indicate standard errors. Gel: gellan gum coating (0.5% w/v); G1: 1.2 μL/mL of geraniol; G2: 2.4 μL/mL of geraniol; PE1: 360 μg/mL of pomegranate extract; PE2: 720 μg/mL of pomegranate extract.

psychrophilic bacteria, neither at day 0, nor in the rest of the storage period. Strawberries treated with Gel + G1 did not show an initial reduction on PSY, but the counts were maintained constant until day 2 (4.40 log). Gel + G2 treatment significantly reduced initial counts to 3.66 log CFU/g, also constant until day 2. During storage, samples treated with coatings incorporated with geraniol at both concentrations tested, showed an increment in PSY counts, even though the growth rate in these samples was lower than in the control (reaching by day 7 counts between 5.44 and 5.71 log CFU/g).

3.1.4. Microbiological shelf-life

Gellan-based coatings with no biopreservatives were not able to increase the microbiological shelf-life of the product, with no significant reductions in any of the studied microbial populations. Several studies have evaluated the use of gellan coatings in different fresh-cut fruits. Particularly, Azarakhsh et al. (2013) studied the incorporation of limonene (a cyclic terpene, principal component of citric essential oils) in gellan coatings on the microbial and sensory quality of fresh-cut pineapple during storage. In accordance with our findings, these authors observed a significant growth in pineapple's native microflora during storage time, with no significant differences between microbial counts in uncoated fruits and in those coated with gellan without bioactive compounds. However, they found that gellan coatings with limonene at 0.3 and 0.5% v/v significantly reduced mesophilic bacteria counts compared to untreated pineapple.

In the present study, the incorporation of pomegranate extract into the Gel coatings, at both tested concentrations, did not show effects on native microflora of fresh-cut strawberries compared to control samples. The lack of effectiveness of PE to control the native microflora could be related to the fact that its principal antimicrobial component (ellagic acid) also naturally occurs in strawberries. In a study performed by Williner, Pirovani, and Güemes (2003), strawberries showed significantly higher levels of ellagic acid (0.16–2.07 mg/g dry weight) compared to many other fruits of economic importance, such as tangerines, pears, apples, bananas and peaches (with ellagic acid content of 0.02–0.07 mg/g dry weight). This could cause an adaptation or resistance of the native microflora towards the activity of this compound, reducing the effectiveness of the pomegranate extract in this case, when applied *in vivo* in fresh-cut strawberries.

With respect to geraniol, its incorporation in gellan-based coatings was able to reduce the growth of mesophilic and psychrophilic bacteria, as well as yeast and molds. In another study, Raybaudi-Massilia, Mosqueda-Melgar & Martín-Belloso (2008) evaluated the evolution of native microflora in fresh-cut melon with alginate-based coatings with different bioactive compounds. In this study, alginate coatings with no biopreservatives achieved a significant reduction in the native microflora of fresh-cut melon (mesophilic and psychrophilic bacteria, and yeast and molds), extending the microbiological shelf-life from 4 days in the uncoated control until almost 10 days in samples with alginate coating. These authors evaluated several natural compounds incorporated in the coatings, and in particular, observed an important antimicrobial activity in those alginate coatings incorporated with geraniol at 0.5% v/v (equivalent to 5 μL/mL), extending the microbiological shelf-life of the product to more than 21 days (Raybaudi-Massilia et al., 2008).

Taking into account the maximum limit of mesophilic aerobic total count at expiry date allowed by the Spanish regulation for hygienic processing, distribution and commerce of prepared meals (BOE, 2001), which is 10^7 CFU/g, gellan-based coatings incorporated with geraniol were able to extend the shelf-life of fresh-cut strawberries from a microbiological point of view from 5 days in the uncoated samples to more than 7 days in samples Gel + G1 and Gel + G2.

3.2. Effect of gellan coatings on sensory properties of fresh-cut strawberries

Edible coatings are consumed together with the food product. Therefore, the sensory quality, especially flavor attributes, is essential to determine the success of the EC in the food market. An unpleasant flavor, or a poorly appealing appearance usually affects the consumers acceptability towards the product (Lin & Zhao, 2007; Rojas-Graü et al., 2009).

Scores obtained in the sensory analysis of fresh-cut strawberries with gellan coatings are observed in Fig. 4. Initially (t = 0 d), there were no differences between treated and control samples on the evaluated sensory attributes, which indicates that gellan-based coatings could be compatible with MP fruit products and accepted by consumers in terms of flavor and appearance. Only *off-odor* scores were significantly higher in those strawberries coated with Gel + G, which could be due to the fact that geraniol has a characteristic rose-like odor and taste (at 10 ppm, approximately 0.01 μL/mL), which is described as sweet floral rose-like, citrus with fruity, waxy nuances (Burdock, 2009). In this sense, Azarakhsh et al. (2013) also observed a detriment in the sensory characteristics of fresh-cut pineapple coated with gellan when incorporated with limonene, compared to those coatings without the bioactive compound.

By day 7 of storage, an important detriment in the sensory characteristics of the strawberries was observed, in both treated and untreated samples. At this point, taste attributes were not evaluated because of the important microbial load of the product. The most affected parameters in time were *OVQ*, *texture* and *off-odor*. Every sample showed a decrease in *OVQ*, where samples treated with Gel + G1 and Gel + G2 showed the lowest scores. This is due to the important loss of firmness observed in the samples (Fig. 4). The increment in *off-odor* at day 7 could be related to the important microbial load found in the samples. Raybaudi-Massilia et al. (2008) also found a diminishment in firmness of fresh-cut melon through storage time, associated to the products processing, and the damage caused during the fruit cutting, releasing enzymes and substrates, such as pectin methylesterase. Enzymatic reactions may also cause detriment of other sensory attributes, such as production of off-odors and off-taste (Varoquaux & Wiley, 1994).

Samples with gellan-based coatings incorporated with geraniol showed more firmness loss with respect to control than the other treatments (Fig. 4). In accordance with our findings, Raybaudi-Massilia et al. (2008) also observed that those coatings incorporated with bioactives at high concentrations (among them, alginate-based coatings with 0.5% v/v geraniol), diminished the firmness of fresh-cut melon through storage. This result could be a consequence of the action of

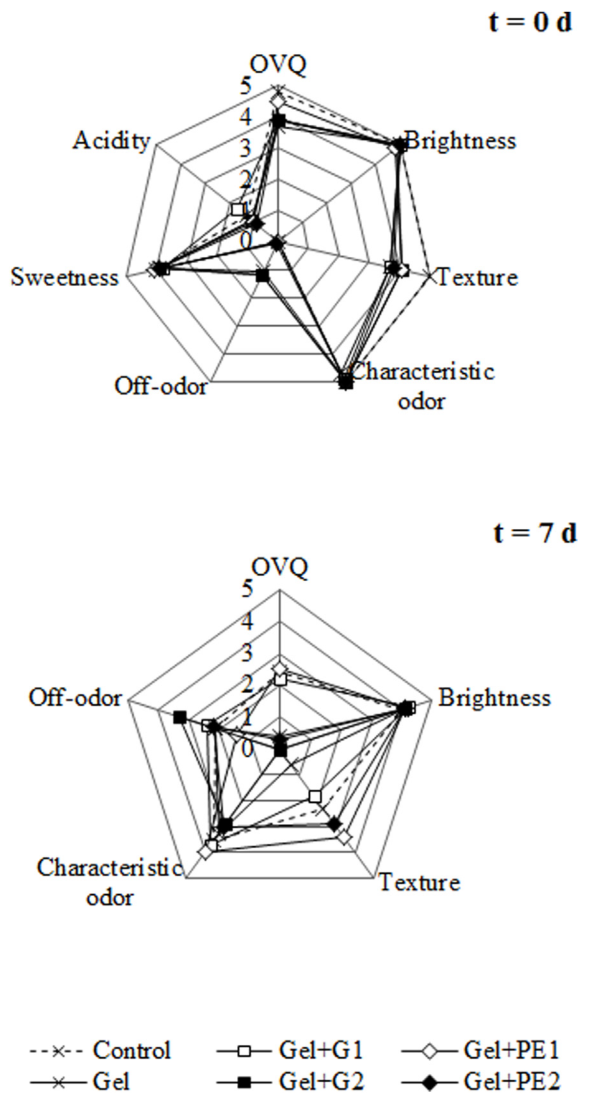

Fig. 4. Effect of gellan-based coatings on sensory characteristics of fresh-cut strawberries through refrigerated storage at 5°C. Sweetness and acidity were not evaluated at 7 days of storage due to the important microbial load of the samples. Gel: gellan gum coating (0.5% w/v); G1: 1.2 μL/mL of geraniol; G2: 2.4 μL/mL of geraniol; PE1: 360 μg/mL of pomegranate extract; PE2: 720 μg/mL of pomegranate extract.

geraniol on the cellular tissue of the fruit, that possibly provokes structural changes that directly affects the firmness, or even increases the release of enzymes and substrates that also favor the softening of the fruit (Raybaudi-Massilia et al., 2008).

4. Conclusions

The incorporation of geraniol at both tested concentrations into gellan-based coatings significantly reduced mesophilic and psychrophilic bacteria, as well as yeast and molds counts, on fresh-cut strawberry compared to control sample. Therefore, gellan-based edible coatings containing geraniol improved the microbiological stability of fresh-cut strawberry in comparison with non-coated product. On the other hand, the incorporation of PE was not able to control the microbial growth of the product. With regards to the sensory analysis, an important firmness loss was found in the control sample through storage time, which neither of the evaluated coatings was able to improve. In fact, samples with gellan-based coatings incorporated with geraniol showed a more important loss of firmness than control.

It is important to highlight that minimally processed fruits and vegetables are highly perishable products, susceptible to microbial and enzymatic deterioration, which has a direct impact on its sensory characteristics. The tested coatings were not able to extend the shelf-life of the fresh-cut strawberries from a sensory point of view, because none was able to improve the texture, one of the key organoleptic parameters in MP products. Therefore, it would be convenient to evaluate different coating formulations, for example, reducing geraniol concentration, or combining different bioactive compounds to reduce the impact on the texture while controlling the native microflora in order to extend the shelf-life of fresh-cut strawberries. Gellan-based coatings as vehicle to carry natural antimicrobials are a promising preservation treatment compatible with organic fresh-like food products such as MP fruits and vegetables.

Conflicts of interest

The authors declare no conflict of interest.

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