Antiviral and antioxidant properties of active alginate edible films containing phenolic extracts

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ABSTRACT

In this work, novel active edible films having antiviral activity were developed through the solvent casting method. Emulsified edible films were prepared by adding lipids into alginate films in order to improve their water vapour permeability. In addition, two natural extracts rich in phenolic compounds, specifically a green tea extract (GTE) and a grape seed extract (GSE) were used as active agents. Interestingly, incorporating GTE or GSE within the biopolymer matrix, the films exhibited significant antiviral activity against murine norovirus (MNV) and hepatitis A virus (HAV) since ~2 log reduction were recorded for the 0.75g extract/g alginate in the film, being GTE more efficient than GSE. Furthermore, the incorporation of phenolic compounds imparted antioxidant capacity to the active films, showing 14.79 ± 0.25 and 3.50 ± 0.25 mM Trolox· g extract in the film⁻¹ for 0.75 GTE and 0.75 GSE films, respectively. It was also found that water barrier efficiency improved upon addition of the highest amount of GTE or GSE (1:0.75 alginate:extract ratio) which was ascribed to molecular interactions between the films' components, as demonstrated by infrared spectroscopy assays.

These results reveal the potential of these active films, mainly the ones containing GTE, to improve food quality and safety.

KEYWORDS: Enteric viruses, Active Packaging, Edible Films, Grape Seed Extract (GSE), Green Tee Extract (GTE).

1 INTRODUCTION

There is an increasing interest in the application of edible coatings and films in the food industry, motivated by the increasing consumer demand for safe, stable foods, and the consciousness of the undesirable environmental effects of non-biodegradable packaging.

Edible coatings are particularly interesting in food preservation since they have demonstrated the ability of improving food quality and safety by controlling water vapour, gases and aroma transfer in food systems (Acevedo-Fani, Salvia-Trujillo, Rojas-Grau, & Martín-Belloso, 2015).

Edible films, which contain both polysaccharide and lipid compounds generally have good mechanical and water barrier properties, which can be either generated as a bilayer structure or dried emulsion (Fabra et al., 2016, Phan The et al., 2009). Emulsified edible films may be advantageously used to improve barrier properties and they can be obtained in a one-step process (Fabra et al., 2009a, Hambleton et al., 2009, Jiménez et al., 2012). Polysaccharides generally form films with good mechanical properties but their hydrophilic nature makes them to have poor barrier properties. Conversely, lipids give rise to good moisture barrier.

Incorporation of antimicrobials has also become popular to prepare active films and coatings since the growth of spoilage or pathogenic microorganisms is one of the major problems affecting food quality and safety. Extensive research has been conducted to include in the film or coating natural antimicrobials such as essential oils (Artiga-Artigas et al., 2017, de Oliveira et al., 2017, Moghimi et al., 2017, Yousuf and Srivastava, 2017, Randazzo et al., 2016), bacteriocins and phenolic compounds (Alkan and Yemenicioğlu, 2017, Cheng et al., 2015). However, although their bactericide and fungicide properties have been broadly investigated, little information is available in the literature about how biopolymers could act

as carriers of antiviral compounds and how they interact with edible film or coating's components.

In this regard, antiviral edible coatings are a promising area to explore due to the risk of disease transmission by food contaminated with enteric viruses, most notably human norovirus (HuNoV) and hepatitis A virus (HAV). Globally, HuNVs is responsible of more than 120 million cases and 35,000 deaths annually attributed to food while HAV causes 14 million cases, being 28,000 cases annually attributed to food (WHO, 2015). Furthermore, edible coatings with antiviral properties could provide an added value, as they could be designed to inactivate viruses, which are normally more resistant than bacteria (Martínez-Abad et al., 2013). For instance, they could play an important role in raw and minimally processed fruits and vegetables which are typically sold to the consumer in a ready-to-eat form and, thus, they do not usually contain preservatives or antimicrobial substances and they are not normally heat processed prior to consumption. More specifically, active coatings having antiviral activity could be crucial for improving food safety of ready-to-eat foods that could be contaminated (I) during cultivation by contact with regenerated irrigation water, (II) during harvesting by infected hand-keepers, and (III) in post-harvest during washing steps with contaminated water.

Natural compounds with demonstrated antiviral activity such as green tea extract (GTE) (Falcó et al., 2018, Randazzo et al., 2017) or grape seed extract (GSE) (Li et al., 2012, Joshi et al., 2015) could be a good choice for their application in the food industry. Existing research works aimed at the use of phenolic compounds in active packaging applications, have been focused on studying their antioxidant (Alkan and Yemenicioğlu, 2017, Cheng et al., 2015, Li et al., 2014) and antibacterial properties (Kanmani, and Rhim, 2014ab). However, from the best of our knowledge, no information exists on the use of these

compounds to develop antiviral films or coatings. Their inclusion into hydrocolloids matrices and the analysis of the performance of these extract-containing materials is of outmost importance, as the efficacy of these polyphenolic compounds may be altered by molecular interactions with the matrices, which could affect the availability of the active compounds. This paper reports, for the first time, the antiviral properties of edible films containing phenolic-rich natural extracts obtained by the emulsion casting method. The morphology, water vapor, optical and active properties in terms of antiviral and antioxidant activity were determined.

2 MATERIALS AND METHODS

2.1 Materials

Alginic acid sodium salt from brown Algae (medium viscosity), soybean oil, oleic acid (technical grade 90%) and Tween 80 were purchased from Sigma-Aldrich. Polyglycerol esters of edible fatty acids (PGE) (E-475) were kindly supplied by Savannah Surfactants, Ltd. (Kundaim, India). GSE and GTE were kindly donated by Optipure (USA) and Naturex, S.A. (France), respectively.

2.2 Development and characterization of active edible films

Six different edible films based on alginic acid sodium salt, surfactants, lipids and extracts were prepared. 1 g of alginic acid (A) sodium salt was directly dissolved in 100 mL of water at room temperature (RT). Once it was completely dissolved, a controlled amount of GTE or GSE were added to obtain 1:0.5 or 1:0.75 alginate:extract ratios. Separately, 0.25g of oleic acid and 0.25 g of soybean were mixed, under magnetic stirring with 0.15g % (w/w) esters of edible fatty acids (PGE) and 0.2g Tween 80, at RT. Both PGE and Tween 80 were needed

for emulsion stabilization. Afterwards, the dissolved alginate solution was added to the lipid/PGE/Tween80 mixture. Dispersions were homogenised for 2 min at 13.500 rpm at RT using a rotor-stator homogenizer (D9, MICCRA GmbH, Müllheim, Deutschland) and, subsequently, the film-forming dispersions were degasified at RT with a vacuum pump. Control films without the extracts were also prepared for comparative purposes.

Films were obtained by the solvent casting method, as previously reported in other works (Fabra et al. 2009ab). Specifically, film-forming dispersions containing 2.3 g of total solids were homogeneously spread over a Teflon plate of 15 cm in diameter and were left for approximately 24 h at 45% RH and 30 °C on a levelled surface until completely dried. These conditions were established after previous experiments to ensure that homogeneous and continuous films without cracks and/or pinholes were obtained. Dried films were peeled intact from the casting surface.

Edible films' nomenclature was 'A' for control alginate film (without lipid), 'A-L' for those containing lipids (without the extract) and 'ab' for those containing the extracts, where 'a' refers to the extract concentration (0.5 or 0.75%), and 'b' is the type of extract (GTE or GSE).

2.2.1 Film conditioning

Before characterization, films were conditioned for two days in a desiccator at 25 °C and 53 % RH, by using oversaturated solutions of magnesium nitrate-6-hydrate (Panreac Química, SA, Castellar del Vallés, Spain).

2.2.2 Scanning electron microscopy

The morphology of the samples was analysed by means of a Scanning Electron Microscope (Hitachi S-4800). Three different samples of each film were frozen in liquid N₂ and randomly

broken to explore the cross-section of the samples. Films were mounted on M4 Aluminium Specimen Mount and fixed on the support using double-side adhesive tape. Then, samples were gold–palladium coated and observed using an accelerating voltage of 5 kV and a working distance of 10 mm.

2.2.3 Optical properties

The optical properties (transparency and color coordinates) were analyzed, in triplicate, by measuring the reflection spectrum of the samples from 400 to 700 nm using a spectrophotometer, model CM-3600d (Minolta CO., Tokyo, Japan), using D65 illuminant/10 observer and an illuminated sample area of 10 mm. Measurements were taken on the side of film which was in contact with air during the drying.

The transparency of the films was determined by applying the Kubelka-Munk theory of a multiple dispersion of reflection spectrum, given the reflection spectra of both black and white backgrounds. Internal transmittance (T_i) of the films was quantified as previously reported by Fabra et al., 2009b. Moreover, CIE- L^* a^* b^* coordinates (CIE, 1986) were obtained from the reflectance of an infinitely thick layer of the material.

2.2.4 Water vapour permeability (WVP)

WVP of the films was determined according to the ASTM E96/E96M-10 (ASTM 2010) gravimetric method and using Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium). Prior to the test, the thickness of the samples was randomly measured at five points. Film samples (35 mm diameter) were secured on Payne permeability cups (Elcometer SPRL, Hermelle/s Argenteau, Belgium), with the outwards-facing side in contact with the air during drying. Before securing the films, 5 mL of oversaturated solution

of sodium chloride (Panreac Quimica, SA) was placed in each cup to expose the film to 75% RH on one side and then, each cup was placed in an equilibrated relative humidity cabinet at 0% RH with dried silica gel and at 25°C. The cabinets were stored at 23 ± 2 °C, weighing the cups periodically (± 0.00001 g) every 2 h, until steady state had been reached. Cups with aluminium samples were used as control samples to estimate solvent loss through the sealing. Water vapour permeation rate was calculated from the steady-state permeation slopes obtained from the regression analysis of weight loss data vs. time, once the steady state had been reached, divided by the film area and, the weight loss was calculated as the total cell loss minus the loss through the sealing. Permeability was obtained by multiplying the permeance by the average film thickness.

2.2.5 Fourier transform infrared (FT-IR) analysis of the materials

The attenuated total reflectance (ATR)-FTIR spectra of the developed films were obtained in a Thermo Nicolet Nexus equipment. The acquisition time was 128 s at 4 cm^{-1} resolutions, and the average spectra in the $4000\text{--}400\text{ cm}^{-1}$ range are reported.

2.2.6 Antioxidant activity

The antioxidant capacity of active films and natural extracts (GTE and GSE) was evaluated by means of the Trolox equivalent antioxidant capacity (TEAC), using a modification of the original TEAC method (Re et al. 1999). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as antioxidant standard. Each film sample of 12 ± 2 mg was cut into small pieces and hydrated with 2 mL of distilled water for two hours, and then, 8 mL of methanol were added in order to favour the extraction of the natural extracts. The supernatant (film extract) obtained was analysed for ABTS radical scavenging activity. To this end, 10

191 μL of the film extracts were added to 1 mL of the ABTS⁺ solution, and absorbance at 734
192 nm was registered every minute for 6 min. For calibration, Trolox standards of different
193 concentrations were prepared, and the same procedure was followed. The TEAC of the film
194 samples was determined by comparing the corresponding percentage of absorbance reduction
195 at 6 min with the Trolox concentration–response curve. All the determinations were carried
196 out in triplicate using a Beckman Coulter DU 730 (England) spectrophotometer, with
197 methanol as the blank.

199 **2.2.7 Determination of antiviral activity**

200 Murine norovirus (MNV-1) was propagated and assayed in RAW 264.7 cells (both kindly
201 provided by Prof. H.VW. Virgin, Washington University School of Medicine, USA), while
202 hepatitis A virus (HAV) strain HM-175/18f (purchased from ATCC VR-1402) in FRhk-4
203 cells (kindly provided by Prof. A. Bosch, University of Barcelona, Spain).

204 Pieces of each edible film (25 ± 5 mg) were sterilized with UV light in a safety cabinet under
205 laminar flow for 15 min and then mixed with 500 μL of MNV and HAV suspensions diluted
206 in PBS pH 7.2 (*ca.* 5 logs TCID₅₀/ml). The samples were incubated overnight at 37°C in a
207 shaker (180 rpm). Then, the effect of the active compound was neutralized with Dulbecco's
208 Modified Eagle Medium (DMEM, Biowest) supplemented with 10% fetal calf serum (FCS).
209 Each treatment was performed in triplicate. Confluent RAW 264.7 and FRhK-4 monolayers
210 in 96-well plates were used to evaluate the antiviral effect of the edible films. Positive
211 controls were virus suspensions incubated with alginate films without GTE or GSE under the
212 same experimental condition. The decay of MNV and HAV titers was calculated as $\log_{10}$
213 (N_x/N_0), where N_0 is the infectious virus titer for alginate films and N_x is the infectious virus
214 titer for alginate films containing GTE or GSE (Falcó et al., 2018).

2.2.8 Statistical analysis

The statistical analysis of data was carried out by means of IBM SPSS Statistics software (v.23) (IBM Corp., USA) through the analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) was used at the 95% confidence level for multiple comparison tests.

3. RESULTS AND DISCUSSION

Edible films and coatings have to accomplish several aspects for successfully being applied in a food product. Alginate pure films even having active properties (either antimicrobial or antioxidant) have limited applications due to their solubility at medium-high relative humidity conditions or products with high water-activity values (i.e. postharvest, meat products, etc.). In this regard, barrier properties of hydrophilic matrices such as alginate have to be improved by adding lipids in order to counteract, for instance, water vapor transfer properties. Oleic acid and soybean oil were chosen as being hydrophobic, generally recognized as safe (GRAS) compounds and, screening studies were carried out to maximize the charge of lipids in the continuous hydrocolloid matrices. To this end, the PEG and Tween 80 were used as surfactants that impart stability to the emulsion and the amount of each compound was previously optimized. However, all these compounds will have an impact on the morphology, barrier and active properties in light of possible interactions between films' compounds, which have been thoroughly analyzed in this paper.

3.1. Morphology and optical properties

The resulting microstructure of the edible films obtained by the solvent casting method is affected by the internal organization of the different components in the emulsion, and by how

they interact during the drying process. Thus, the morphological analysis offers relevant information about the arrangement of the components in the films. Figure 1 displays representative SEM micrographs of the cross-section images of the developed films, where remarkable differences can be observed. The pure alginate film exhibited a smooth and homogeneous appearance, suggesting the formation of an ordered matrix. In contrast, a coarse aspect can be distinguished when oleic acid, soybean oil and the surfactant PGE were incorporated into the polysaccharide matrix.

Interestingly, two differentiated phases of lipid-rich and alginate-rich phases can be distinguished in the control films (A-L), indicating that lipid compounds mostly migrated to the surface during the drying process, fact which had a positive impact on barrier properties as it will be shown below. A more heterogeneous matrix was observed when phenolic extracts (either GTE or GSE) were incorporated into the hydrocolloid matrix, where the lipid compounds appear randomly distributed throughout the film cross-section, and no lipid phase separation was clearly discerned. This effect could be ascribed to the existing interactions between the phenolic extracts and the lipid fraction as it will be described below.

As it has been previously reported, the transparency of the films is directly linked with the internal structure of the developed films (Fabra et al., 2009ab). Thus, the effect of lipid and phenolic extracts addition on the neat alginate films was quantitatively assessed by means of the internal transmittance (T_i) measurements. Figure 2 displays the spectral distribution curves of T_i , which clearly depends on the internal microstructure of the matrix, the presence of natural extracts and the distribution of the films' components. The presence of more heterogeneous structures promotes light dispersion and a reduction of T_i . The presence of dispersed lipids in the alginate matrix slightly reduced the transparency of the edible films

due to an increase in light scattering. In the case of natural extracts, the absorbance of GSE and GTE also contributed to lowering the transmittance (being greater in those containing GSE), indicating that these films were more opaque and heterogeneous than their counterparts prepared without the extracts. These effects can be explained by the light selective absorption of polyphenols present in the extracts at low wavelengths that imparts a reddish color to the films, thus decreasing the hue and T_i values at low wavelengths. An increase in film opacity because of the addition of antioxidant has also been reported in chitosan films incorporated with GTE (Siripatrawan and Harte, 2010) or in agar and carrageenan films incorporated with GSE (Kanmani and Rhim, 2014ab). The color parameters (lightness $-L^*$ -, chrome $-C^*$ - and hue $-h^*$ -), listed in Table 1, evidence that the incorporation of natural extracts, either GTE or GSE, led to darker (lower L^*) and more reddish (lower h^*) edible films, being this effect more obvious for GSE. In general, the addition of GTE gave rise to a more saturated color in the films (higher C^*), whereas GSE provided less vivid films (lower C^*).

3.2 Water Vapor Permeability (WVP)

Table 2 gathers water vapor permeability values of the developed films. As expected, pure alginate films exhibited the highest water vapor permeability and the incorporation of lipid mixtures greatly reduced WVP values in approximately 42.5 %. Interestingly, despite being mostly hydrophilic compounds, incorporation of both extracts led to improve barrier properties of the developed edible films. This effect can be ascribed to the presence of polyphenols which can form hydrogen bonds and hydrophobic interactions with the polar groups in the lipids, thus, limit the amount of free OH groups which could interact with water. Supporting this hypothesis, interactions between green tea catechins and milk fat globules

(Rashidinejad et al., 2017) or with lipid bilayers of cell membranes (Sirk et al., 2008) have been previously reported.

However, some controversial data have been reported in the literature. Whereas Hong et al. (2009) reported that the water vapor barrier efficiency of agar based films increased by the addition of high concentrations of GTE or GSE, other authors found that the incorporation of GSE at concentrations up to 0.66g extract/g polysaccharide increased the water vapor permeability of agar-based films (Kanmani and Rhim, 2014), *Gelidium corneum*/nano-clay composite films (Lim et al., 2010) or rapeseed protein/gelatin films (Jang et al., 2011). These latter authors ascribed the increase of WVP to the loss of intermolecular interactions among the film matrix and the increase of film porous size by GSE addition. It should be mentioning that, as compared to data reported in these works, lipids were also present in the alginate films developed in this study contributing to improve barrier properties. In fact, as it can be seen in Table 2, a significant reduction in the WVP was only observed when the highest extract concentration was used, indicating that molecular interactions were accentuated at this concentration.

3.3 Molecular interactions

Fourier transform infrared spectroscopy (FTIR) was used in order to investigate potential interactions between matrix components. FTIR spectra of neat compounds and blend films in the presence and absence of the highest concentration of GTE or GSE are gathered in Figure 3. The spectrum of control films (A-L) showed characteristic peaks of alginate and lipid compounds. The broad absorption band between 3000-3600 cm⁻¹ is assigned to -OH stretching of bulk water and thus, the shifts are attributed to water content changes and water-

matrix interactions which could affect the network organization and no further discussion can be provided about the interaction between extracts and lipids in this region.

When incorporating natural extracts, the contribution of the C=O group of triglycerides with a stretching vibration band at approximately 1740 cm^{-1} slightly shifted to higher wavenumbers, mainly by the presence of GTE. Similarly, the small shoulder at 1710 cm^{-1} assigned to free fatty acids (Guillén and Cabo, 1997) also shifted towards higher wavenumbers by the presence of GTE. It is worth mentioning that shifts in the bands associated with PO_2^- stretching vibrations (at 1238 cm^{-1}), mainly observed in those containing GTE were also observed which can be ascribed to changes that occur in the polar head group regions of the lipid compounds. Similarly, Gaudreau et al. (2016) observed that the presence of tea extract modified the bacterial lipid structure and composition. These PO_2^- groups can be associated to the presence of soybean phospholipids in the soybean oil. However, some authors also assign the band at 1238 cm^{-1} to bending vibration out-of-plane of the methylene group (Safar et al., 1994).

The bands between 1500 and 1000 cm^{-1} are the most difficult to assign. At approximately 1465 cm^{-1} , a shift in the band associated to the bending vibration of the methylene group (Guillen and Cabo, 1997) occurred in the presence of GSE. Differences in twisting and wagging vibrations of the $-\text{CH}_2$ groups are noticeable in the zone between 1350 and 1150 cm^{-1} in the presence of the extracts, mainly GTE. In agreement with these findings, Rashidinejad et al., 2016 have recently demonstrated the presence of hydrophobic interactions between green tea catechins and milk fat globules surfaces.

Thus, the FTIR characterization allowed to identify the groups involved in the interactions between the phenolic extracts and the films' components, and confirmed the assumptions from the functional properties. These interactions could also be related to the antioxidant and

antimicrobial properties, since the availability of the phenolic extracts could be limited to some extent.

3.4 Antioxidant activity

Table 3 shows the antioxidant activity of the developed alginate-based films determined by the ABTS assay, which evaluates the capacity of the films to scavenge free radicals. Data are expressed as mmolTrolox/g antioxidant (TEAC values), by considering the antioxidant mass fraction in the dry sample.

The antioxidant activity of the pure GTE and GSE expressed, as mmol Trolox per g antioxidant were 20.1 ± 0.41 and 5.83 ± 0.37 respectively, which were higher than those found in the literature for tea extracts and similar for GSE extracts (Magwebeba et al., 2016, Majchzak et al., 2004). The first clear observation is that TEAC values of the active edible films referred to the extract content were significantly lower than those obtained for pure GSE or GTE extracts. This means that the GTE and GSE in the active edible films had lower antioxidant capacity, probably due to the existing interactions between the polyphenols with other films' components, preventing a complete release of the antioxidant components from the film structure. Furthermore, the antioxidant activity of the films significantly increased ($p \leq 0.05$) with increasing GTE or GSE concentration as shown in Table 4. It is worth mentioning that alginate/lipid films without phenolic extracts (GTE or GSE) did not show any antioxidant activity. These findings agree with those previously reported for grape seed extract (Moradi et al., 2012) or green tea extract (Siripatrawan & Harte, 2010) who observed an increase in the scavenging activity of the chitosan-GTE or GSE films with increasing extract concentration.

These results suggest that the incorporation of natural phenolic extracts into alginate/lipid films improved barrier properties and imparted antioxidant activity to the films. Interactions between lipid compounds and polyphenolic compounds from GTE or GSE may play an important role on the film properties as it has been discussed further based on the FTIR data obtained in the section 3.3. The existing interactions contribute, on the one hand, to reduce the water vapor permeability but it also affects the antioxidant capacity of the edible films as compared to the neat phenolic extracts based on the availability of the extracts.

3.5 Antiviral properties of alginate films

The antiviral effect of GTE and GSE against human norovirus surrogates and HAV has been previously reported (Su and D'Souza, 2011, Su and D'Souza, 2013, Randazzo et al., 2017, Falco et al., 2018), suggesting the potential to be incorporated in active packaging to control enteric viruses. In the current study, alginate films containing GTE and GSE at 0.75 g extract/g alginate decreased MNV titers by 1.92 and 1.67 log TCID₅₀/mL, respectively (Figure 4A). Films containing 0.50 g extract/g alginate, reduced MNV titers by 2.00 and 0.96 log TCID₅₀/mL for GTE and GSE films respectively (Fig 4A). HAV titers decreased by 1.92 and 1.50 when treated with GTE and GSE with 0.75g extract/g alginate, respectively, while 1.25 and 1.38 log reduction were recorded at lower GTE and GSE concentrations, respectively (Fig 4B).

From the results obtained, one can deduce that active films showed lower antiviral activity than the pure extracts (Falco et al., 2018 and Su and D'Souza, 2013), suggesting that part of the GTE or GSE could interact between film's components, preventing a complete release of the active compounds, accordantly with the results obtained for the antioxidant properties.

Interestingly, these results showed that alginate films containing GTE were slightly more efficient on HAV and MNV than their counterparts containing GSE. Furthermore, the antiviral activity generally increased as the concentration of phenolic extracts increased in the film, except in the case of GTE where its efficacy against MNV were similar at both GTE concentrations. While the antibacterial activity of GTE and GSE has been widely reported, including their use in food applications (reviewed by Perumalla, and Hettiarachchy, 2011; Tavassoli-Kafrani, et al., 2016), very limited information is available about studies evaluating films with antiviral properties. So far, only few antimicrobials, such as silver, copper and cinnamaldehyde, have been proved to exert antiviral activity against norovirus surrogates (feline calicivirus and MNV) and HAV when incorporated within biopolymeric materials, resulting in a very efficient technology for controlling viral contamination on food contact surfaces (Castro-Mayorga et al., 2017; Fabra et al. 2016; Martínez-Abad et al., 2013). To the best of our knowledge, only one similar research work reports the antiviral activity of these extracts when incorporated within biopolymeric material (Amankwaah (2013)). In this study, chitosan films loaded with GTE and GSE reduced MNV infectivity by 1.60 and 1.90 log PFU/ml after 24 h contact with 5 % GTE and GSE films, respectively (Amankwaah, 2013). However, it should be stressed that a synergic effect could have been observed, as chitosan has also proved to have antiviral activity against human norovirus surrogates (Su et al., 2009)

4. CONCLUSIONS

Antiviral films against MNV and HAV were prepared by incorporating either GTE or GSE as natural antimicrobial agents into alginate-lipid films to be used as active edible films. The

extract-containing alginate-lipid films exhibited strong antioxidant and antiviral activity against the most widely used human norovirus surrogate, i.e. MNV, and HAV, although the GTE tested in this work showed better antiviral and antioxidant properties than GSE. Interestingly, the water vapor permeability was improved probably due to intermolecular interactions between the films' components. However, remarkable changes in transmittance and color parameters were observed in edible films containing the phenolic compounds. The active edible films prepared in this work, mainly those containing GTE, exhibited interesting properties to improve food safety and quality of ready-to-eat foods.

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REFERENCES

- ASTM 2010. Standard test methods for water vapor transmission of materials, Vol. ASTM E96/E96M-10, ASTM International, West Conshohocken, PA (2010).
- Acevedo-Fani, A., Salvia-Trujillo, L., Rojas-Graü, M.A., Martín-Belloso, O. (2015). Edible films from essential-oil-loaded nanoemulsions: Physicochemical characterization and antimicrobial properties. *Food Hydrocolloids*, 47, 168-177.
- Alkan, D., Yemenicioğlu, A. (2016). Potential application of natural phenolic antimicrobials and edible film technology against bacterial plant pathogens. *Food Hydrocolloids*, 55, 1-10

428 Artiga-Artigas, M., Acevedo-Fani, A., Martín-Belloso, O. (2017). Improving the shelf life of
 429 low-fat cut cheese using nanoemulsion-based edible coatings containing oregano essential
 430 oil and mandarin fiber. *Food Control*, 76, 1-12.

431 Cheng, S.-Y., Wang, B.-J., Weng, Y.-M. (2015) Antioxidant and antimicrobial edible
 432 zein/chitosan composite films fabricated by incorporation of phenolic compounds and
 433 dicarboxylic acids. *LWT - Food Science and Technology*, 63(1), 115-121

434 De Oliveira, K.Á.R., Berger, L.R.R., de Araújo, S.A., Câmara, M.P.S., de Souza, E.L. (2017).
 435 Synergistic mixtures of chitosan and *Mentha piperita* L. essential oil to inhibit *Colletotrichum*
 436 species and anthracnose development in mango cultivar Tommy Atkins. *Food Microbiology*,
 437 66, 96-103.

438 Fabra, M.J., Flores-López, M.L., Cerqueira, M.A., Jasso de Rodríguez, D., Lagaron, J.M.,
 439 Vicente, A.A. (2016) Layer-by-Layer Technique to Developing Functional Nanolaminate
 440 Films with Antifungal Activity. *Food and Bioprocess Technology*, 9(3), 471-480

441 Fabra, M.J., Jiménez, A., Atarés, L., Talens, P., Chiralt, A. (2009a). Effect of fatty acids and
 442 beeswax addition on properties of sodium caseinate dispersions and films.
 443 *Biomacromolecules* 10 (6), 1500–1507

444 Fabra, M.J., Talens, P., Chiralt, A. (2009b). Microstructure and optical properties of sodium
 445 caseinate films containing oleic acid-beeswax mixtures. *Food Hydrocolloids*, 23, 676-683.

446 Falcó, I., Randazzo, W., Gómez-Mascaraque, L., Aznar, R., López-Rubio, A., Sánchez, G.,
 447 (2018). Fostering the antiviral activity of green tea extract for sanitizing purposes through
 448 controlled storage conditions. *Food Control* 84, 485-492

449 Gaudreau, H., Champagne, C.P., Remondetto, G.E., Alvarez, P.A., Gomaa, A., Subirade, M.
 450 (2016). Tea extract render probiotic *Lactobacillus helveticus* more resistant to oxygen
 451 exposure through lipid modification mechanism. *Food Research International*, 81, 141-148

452 Guillén, M.D., Cabo, N. (1997). Characterization of edible oils and lard by fourier transform
453 infrared spectroscopy. Relationships between composition and frequency of concrete bands
454 in the fingerprint region. *JAACS, Journal of the American Oil Chemists' Society*, 74 (10),
455 1281-1286.

456 Hambleton, A., Fabra, M.J., Debeaufort, F., Dury-Brun, C., Voilley, A. (2009). Interface and
457 aroma barrier properties of iota-carrageenan emulsion-based films used for encapsulation of
458 active food compounds. *Journal of Food Engineering*, 93, 80-88.

459 Hong, Y. H., Lim, G. O., & Song, K. B. (2009). Physical properties of Gelidium corneum-
460 gelatin blends films containing grapefruit seed extract or green tea extract and its application
461 in the packaging of pork loins. *Journal of Food Science*, 74(1), C6–C10.

462 Jang, S. A., Shin, Y. J., & Song, K. B. (2011). Effects of rapeseed protein-gelatin film con-
463 taining grapefruit seed extract on ‘Maehyang’ strawberry quality. *International Journal of*
464 *Food Science and Technology*, 46, 620–625.

465 Jiménez, A., Fabra, M.J., Talens, P., Chiralt, A (2012). Effect of re-crystallization on tensile,
466 optical and water vapour barrier properties of corn starch films containing fatty acids. *Food*
467 *Hydrocolloids*, 26, 302-310.

468 Joshi, S.S., Su, X., and D'Souza, D.H. (2015). Antiviral effects of grape seed extract against
469 feline calicivirus, murine norovirus, and hepatitis A virus in model food systems and under
470 gastric conditions. *Food Microbiology*, 52, 1-10.

471 Kanmani, P. and Rhim, J.W. (2014a). Antimicrobial and physical-mechanical properties of
472 agar-based films incorporated with grapefruit seed extract. *Carbohydrate Polymers*, 102,
473 708-716.

474 Kanmani, P. and Rhim, J.W. (2014b). Development and characterization of
 475 carrageenan/grapefruit seedextract composite films for active packaging. *International*
 476 *Journal of Biological Macromolecules*, 68, 258–266.

477 Li, D., Baert, L., Zhang, D., Xia, M., Zhong, W., Van Coillie, E., Jiang, X., Uyttendaele, M.,
 478 2012. Effect of grape seed extract on human norovirus GII.4 and murine norovirus 1 in viral
 479 suspensions, on stainless steel discs, and in lettuce wash water. *Appl. Environ. Microbiol.*
 480 78, 7572-7578.

481 Li, J.H., Miao, J., Wu, J.L., Chen, S. F., Zhang, Q.Q. (2014). Preparation and characterization
 482 of active gelatin-based films incorporated with natural antioxidants. *Food Hydrocolloids*, 37,
 483 166-173.

484 Lim, G. O., Jang, S. A., & Song, K. B. (2010). Physical and antimicrobial properties of
 485 Gelidium corneum/nano-clay composite film containing grapefruit seed extractand thymol.
 486 *Journal of Food Engineering*, 98, 415–420

487 Magcwebeba, T.U., Riedel, S., Swanevelder, S., Swart, P., De Beer, D., Joubert, E.,
 488 Christoffel, W., Gelderblom, A. (2016). The potential role of polyphenols in the modulation
 489 of skin cell viability by *Aspalathus linearis* and *Cyclopia spp.* Herbal tea extracts *in vitro*.
 490 *Journal of Pharmacy and Pharmacology*, 68, 1440-1453.

491 Majchrzak, D., Mitter, S., Elmadfa, I. (2004). The effect of ascorbic acid on total antioxidant
 492 activity of black and green teas. *Food Chemistry*, 88(3), 447-451.

493 Martínez-Abad, A., Ocio, M.J., Lagarón, J.M., Sánchez, G. (2013). Evaluation of silver-
 494 infused polylactide films for inactivation of Salmonella and feline calicivirus in vitro and on
 495 fresh-cut vegetables. *International Journal of Food Microbiology*, 162(1), 89-94

496 Moghimi, R., Aliahmadi, A., Rafati, H. (2017). Antibacterial hydroxypropyl methyl cellulose
 497 edible films containing nanoemulsions of Thymus daenensis essential oil for food packaging

498 (2017) Carbohydrate Polymers, 175, 241-248.

499 Moradi, M., Tajik, H., Razavi Rohani, S. M., Oromiehie, A. R., Malekinejad, H., Ali-akbarlu,
500 J., & Hadian, M. (2012). Characterization of antioxidant chitosan film incorporated with
501 Zataria multiflora Boiss essential oil and grape seed extract. *LWT—Food Science and*
502 *Technology*, 46, 477–484.

503 Phan The, D., Debeaufort, F., Voilley, A., Luu, D. (2009) Influence of hydrocolloid nature
504 on the structure and functional properties of emulsified edible films. *Food Hydrocolloids*,
505 23(3), 691-699

506 Perumalla, A.V.S., Hettiarachchy, N.S. (2011). Green tea and grape seed extracts - Potential
507 applications in food safety and quality. *Food Research International*, 44(4), 827-839

508 Randazzo, W., Falcó, I., Aznar, R., Sánchez, G. (2017). Effect of green tea extract on enteric
509 viruses and its application as natural sanitizer. *Food Microbiology*, 66, 150-156.

510 Randazzo, W., Jiménez-Belenguer, A., Settanni, L., Perdonés, A., Moschetti, M., Palazzolo,
511 E., Guarasi, V., Vargas, M., Germanà, M.A., Moschetti, G. (2016). Antilisterial effect of
512 citrus essential oils and their performance inedible film formulations. *Food Control*, 59, 750-
513 758.

514 Rashidinejad, A., Birch, E.J., Everett, D.W. (2016). Interactions between milk fat globules
515 and green tea catechins. *Food Chemistry*, 199, 347-355.

516 Rashidinejad, A., Birch, E. J., Hindmarsh, J., Everett, D. V. (2017) Molecular interactions
517 between green tea catechins and cheese fat studied by solid-state nuclear magnetic resonance
518 spectroscopy. *Food Chemistry*, 215, 228-234.

519 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999).
520 Antioxidant activity applying an improved ABTS radical cation decoloration assay. *Free*
521 *Radical Biology and Medicine*, 26, 1231-1237.

Safar, M., D. Bertrand, P. Robert, M.F. Devaux, and C. Genot, Characterization of Edible Oils, Butters and Margarines by Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance, *Ibid.*:371–377 (1994).

Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids*, 24, 770–775.

Sirk, T.W., Brown, E.F., Sum, A.K., Friedman, M. (2008). Molecular Dynamics Study on the Biophysical Interactions of Seven Green Tea Catechins with Lipid Bilayers of Cell Membranes. *Journal of Agricultural and Food Chemistry*, 56, 7750-7758.

Su X, Zivanovic S, D'Souza DH. Effect of chitosan on the infectivity of murine norovirus, feline calicivirus, and bacteriophage MS2. *J Food Prot.* 2009 Dec;72(12):2623-8.

Su, X., D'Souza D.H. (2011). Grape seed extract for the control of human enteric viruses *Applied and Environmental Microbiology*, 77, 3982-3987.

Su, X., D'Souza D.H. (2013). Grape seed extract for foodborne virus reduction on produce. *Food Microbiology*, 34, 1-6

Yousuf, B., Srivastava, A.K. (2017). Flaxseed gum in combination with lemongrass essential oil as an effective edible coating for ready-to-eat pomegranate arils. *International Journal of Biological Macromolecules*, 104, 1030-1038.