Antiviral, antibacterial and antioxidant properties of edible marine polysaccharide-based coatings containing *Larrea nitida* polyphenols enriched extract

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Abstract

The aim of this work was to develop active edible coatings based on marine polysaccharide matrices and polyphenols-enriched native plant extracts from arid and semiarid regions of Argentina. Initially, four plant extracts were characterized in terms of antioxidant, antibacterial and antiviral activity and the one with better biological properties and no toxicity or genotoxicity, *Larrea nitida* (Ln) extract, was incorporated into agar, alginate or agar/alginate matrices. The Ln extract-containing films were characterized in terms of antioxidant, antiviral, physicochemical, antibacterial and polyphenols release performance. The incorporation of Ln extract provided darker films, with a more saturated orange-brownish color and with negligible effects on mechanical and barrier properties. The presence of Ln extract within the polysaccharide matrices reduced the bacterial population of *Listeria innocua* ~ 2.6-3.0 log. Additionally, all the coatings showed antiviral activity when applied to blueberries against murine norovirus (MNV), a cultivable norovirus surrogate. The coatings of agar and Ln extract was able to reduce the infectivity of MNV below the limit of detection after over- night (ON) incubation at 25 ºC and after 4 days at 10 ºC storage.

These edible polysaccharides coatings containing Ln extract could be an alternative to reduce or eliminate food contaminant such as viruses and bacteria and protect the food against oxidative process.

Keywords: Larrea nitida extract, antiviral films, antibacterial films, berries.

1. Introduction

Fresh and minimally processed vegetables and fruits market has grown up exponentially because of the changes in consumer´s lifestyle. Consumer´s preferences towards fresher products with natural additives have made the food industry to focus its efforts on the development of innovative preservation technologies to reduce the growth of both spoilage and foodborne pathogens such as bacteria and human enteric viruses (Dilmaçünal, & Kuleaşan, 2018; Prakash, Baskaran, Paramasivam, & Vadivel, 2018). In this regard, edible coatings have recently emerged as one of the most promising technologies for controlling the quality and safety of fresh products while extending their shelf life (Fabra, Falcó, Randazzo, Sánchez, & López-Rubio, 2018; Falcó et al., 2019; Guo, Yadav, & Jin, 2017; Majid, Nayik, Dar, & Nanda, 2018).

Edible coatings are based on biodegradable, biocompatible and food-grade polymers from natural sources, which include polysaccharides, proteins, and lipids (Hassan, Chatha, Hussain, Zia, & Akhtar, 2018). Among these materials, polysaccharides have been widely studied and used for the development of edible coatings due to good film forming availability as well as suitable mechanical and gas barrier properties (Hou et al., 2019). More concretely, research interest in the use of marine polysaccharides (Kanmani & Whan Rhim, 2014; Tavassoli-Kafrani, Shekarchizadeh, & Masoudpour-Behabadi, 2016; Shankar & Rhim, 2017; Hou et al., 2019) such as carrageenan, agar and alginate as biopolymer matrices has increased as they are highly abundant, inexpensive, absorbent, non-toxic and non-immunogenic (Shankar & Rhim, 2017; Oliveira Filho et al., 2019, Fabra et al., 2018). Furthermore, polysaccharide-based coatings present a high potential to serve as vehicles to incorporate active compounds with antioxidant and antimicrobial properties, which greatly contribute to extend product shelf life and to reduce the risk of pathogen growth on food surface (Bhardwaj, Alam, & Talwar, 2019). In fact, several

works have been carried out in the last decades dealing with active biopolymer matrices containing essential oils and/or natural extracts (Ganiari, Choulitoudi, & Oreopoulou, 2017; Moghimi, Aliahmadi, & Rafati, 2017). Polyphenolic-enriched plant extracts represent an interesting ingredient for the development of edible coatings, mainly due to their natural origin and phytochemical properties, which allows obtaining active materials with the aim of extending the shelf life and the value to the products (Luchese, Brum, Piovesana, Caetano, & Flôres, 2017; Mir, Dar, Wani & Shah, 2018).

Zuccagnia punctata Cav., *Larrea divaricata* Cav., *Larrea cuneifolia* Cav., *Larrea nitida* Cav., and *Tetraglochin andina* Ciald. are shrubs that grow in arid and semiarid regions of Argentina and have demonstrated biological activities such as antifungal (Butassi et al., 2015; 2018; Moreno et al., 2018 a, b), antibacterial (Zampini, Cudmani, & Isla, 2012), antigenotoxic (Zampini et al., 2008), antioxidant (Álvarez Echazú et al., 2018; Carabajal, Zampini & Isla, 2017; Moreno et al., 2018 a, b, c) and anti-inflammatory (Torres Carro et al., 2017), which have been mainly attributed to their high content in chalcones, flavonoids, phenolic acids, and lignans (Isla et al., 2016; Moreno et al., 2018 a, b, c). However, although several biological properties have been studied, the activity of these plant species against bacteria and virus of interest in the food industry has not been previously described and there is not existing literature on their application as active edible coatings.

Therefore, the main aim of this work was: (i) to evaluate the antioxidant, antimicrobial (antiviral and antibacterial) activity, and toxicity of the extracts obtained from Argentinian medicinal plants; (ii) to investigate the activity and physicochemical properties of edible polysaccharide-based films containing the most bioactive extract; and (iii) to assess the antiviral efficacy when applied onto the surfaces of blueberries at room and refrigeration temperatures.

2. Materials and Methods

2.1. Reagents

Alginic acid sodium salt from brown algae (medium viscosity), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich. Agar was obtained from Hispanagar S.A. (Burgos, Spain) and glycerol was purchased from Panreac- Aplichem. Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA), peptone water and Palcam agar were purchased from Scharlab.

2.2. Plant material

The used plant parts were leaves and stems (aerial parts), according to their traditional use. *Zuccagnia punctata* Cav. (Zp), *Larrea divaricata* Cav. (Ld), and *Larrea cuneifolia* Cav. (Lc) were collected in April 2017 at Amaicha del Valle, Tucumán, Argentina at 2000 meters above sea level (m.a.s.l.). *Larrea nitida* Cav. (Ln) was collected in April 2017 at Vinchina, La Rioja, Argentina at 3485 m.a.s.l. *Tetraglochin andina* Ciald. (Ta) was collected in February 2017 in Huaca Huasi, Tucumán, Argentina (4300 m.a.s.l.). The plants were identified by Dra. Soledad Cuello and voucher specimens (Zp: LIL 605935; Ld: LIL 614299; Lc: LIL 614829; Ln: LIL 615845; Ta: LIL 610669) were deposited at the Herbarium of Fundación Miguel Lillo (Tucumán, Argentina). The samples were dried in a forced air oven at 40 °C and then they were ground.

2.3. Dry extract preparation

The powdered dried plant material (10 g) was macerated in 200 mL of 60° ethanol for 1 h with ultrasonic application five times for 10 min. Extracts were filtered, taken to dryness under reduced pressure and then freeze-dried to obtain the plant dry extracts. The extraction yield was determined. The dry extracts were stored at -20 °C until their use.

2.4. Antimicrobial activity of extracts

The antimicrobial activity of plant extracts was tested against *Listeria innocua* (Spanish Type Culture Collection CECT 910, ATCC 33090), as surrogated strain of the pathogen *Listeria monocytogenes* and against *Escherichia coli* (CECT 434; ATCC 25922), Gram-positive and Gram-negative bacterial models, respectively. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the plant extracts were determined in MHB. The antibacterial activity of the plant extracts was determined using sterile 96-well plates (Wiegand, Hilpert, & Hancock, 2008). 100 mg of each plant dry extract were diluted in 10 mL of DMSO, and then serial dilutions were made up in 290 µL of sterile MHB (100-500 µg/mL). Control samples with DMSO were prepared to check the non-toxicity of DMSO. Overnight (ON) cultures of the microorganisms in stationary phase measured at 600 nm were diluted in Tryptone Soy Broth and incubated at 37 °C until reaching the exponential phase corresponding with an optical density of 0.2 and a bacterial concentration of 10^6 CFU/mL. 10 µL of the inoculum were added in each well and incubated at 37 ºC for 24 h. Depending of the turbidity of the wells, serial dilutions with peptone water were made and plated in petri dishes of MHA. Colonies were counted after incubation at 37 ºC for 24 h. Results were expressed as log of colony forming units per milliliter (log CFU/mL) and the logarithmic reduction value (LRV) was calculated with respect to the control samples. The experiment was carried out in triplicate.

2.5. Antiviral activity of plant extracts

2.5.1. Virus propagation and cell line

MNV-1 (kindly provided by Prof. H. W. Virgin, Washington University School of Medicine, USA) was propagated and assayed in RAW 264.7 (also provided by Prof. H.W. Virgin). Semi-purified stocks were subsequently produced from RAW cells by

centrifugation of infected cell lysates at 660 × g for 30 min. Infectious MNV were enumerated by determining the 50% tissue culture infectious dose ($TCID_{50}$) with eight wells per dilution using the Spearman-Karber method (Pinto, Diez, & Bosch, 1994).

2.5.2. Antiviral effect of plant extracts on MNV

Plant dry extracts were dissolved in ethanol 60% to obtain concentrations of 1 and 10 mg/mL. Each solution was mixed with an equal volume of MNV suspensions (ca. 5 log $TCID_{50}$/mL), followed by incubation at 37 ºC in a shaker for 16 h (ON incubation). Thereafter, Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fecal calf serum was added to stop the reactions. Positive controls were virus suspensions mixed with ethanol 60% under same experimental conditions. Ten-fold dilutions of plant extracts-treated and untreated virus suspensions were inoculated into confluent RAW monolayers in 96-well plates. Then, infectious viruses were enumerated by cell culture assays as described above. Each treatment was run in triplicate. The decay of MNV titers was calculated as log10 (N_x/N_0), where N_0 is the infectious virus titer for untreated samples and Nx is the infectious virus titer for plant extract treated samples (Falcó et al., 2018).

2.6. Antioxidant activity of plant extracts

The antioxidant capacity of plant extracts was evaluated through 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 extract was analyzed for ABTS radical scavenging activity to indirectly determine the amount of the extract released. To this, 17 µL of each extracts were added to 200 µL of the $ABTS^+$ solution, and absorbance at 734 nm was registered every minute for 6 min. For calibration, Trolox standards of different concentrations were prepared, and the same procedure was followed. The TEAC of each extract was

determined by comparing the corresponding percentage of absorbance reduction at 6 min with the Trolox concentration–response curve. All the determinations were carried out in triplicate using a CLARIO star (BMG LABTECH) spectrophotometer, using 50 or 10% ethanol solutions as blanks.

2.7. Toxicity assays

2.7.1. Mutagenic activity. The Ames test

The mutagenic effects of plant dry extracts were evaluated on two *Salmonella Typhimurium* strains (TA98 and TA100). The plate incorporation assay was performed according to Maron & Ames (1983), by adding 0.1 mL of the ON bacterial culture, 0.1 mL of extract at different concentrations (350-1400 µg/plate) and 2 mL of top agar on minimal agar. Negative and positive controls were used simultaneously in each experiment. The positive control was 4-nitro-o-fenilendiamine (10 µL/plate, 1 mg/mL solution) for TA98 and TA100, and the negative control was DMSO (100 µL/plate). The revertant colonies of each plate were counted manually after 48 h of incubation at 37 °C and the mutagenicity relation (His^+ revertant per plate/ His^+ spontaneous revertant) was calculated. The extract was considered mutagenic if the number of revertants per plate was more than twice the number of colonies produced on the solvent control plates (spontaneous revertant frequency) or the mutagenicity relation ≥ 2.

2.7.2. General toxicity assay. *Artemia salina* test

The acute toxicity levels of plant dry extracts were studied using *Artemia salina* as test organism (Svensson, Mathiasson, Martensson, & Bergatröm, 2005). To obtain *A. salina* larvae, its cysts were hatched in artificial seawater (NaCl, $MgCl_2.6H_2O$, Na_2SO_4, $CaCl_2.2H_2O$, KCl). After 24 h of incubation at 25 °C, the phototropic nauplii have hatched and are in their most sensitive state. Between 10 and 12 nauplii were transferred to microplates containing 100 µL of fresh medium and 3 µL of different concentrations of

extract dissolved in DMSO (62.5–1000 µg/mL). Solvent control (DMSO) without extract and a positive control (potassium dichromate) were also assayed. All the plates were incubated for 24 h at 25 °C. Plates were then examined under a magnifying glass and the number of dead (immobile) nauplii in each well was counted. The nauplii were considered immobile if they did not show any forward movement for 10 s. The LC_{50} (concentration that kills 50% of the *A. salina* larvae) was calculated.

2.8. Development of active films

Six different films were obtained by the solvent casting method: three without the extract and three with Ln extract. Films containing Ln were prepared as follows: 1 g polysaccharide (agar, alginate or agar/alginate mixed in a 1:1 ratio) was dissolved in 100 mL of distilled water using a magnetic stirrer for 30 min at a controlled temperature of 80 °C until they were completely dissolved. Then, solutions were cooled to 40 °C and 50 mg Ln extract dissolved in 0.3 g glycerol was added, mixed with stirring. Ln was previously dissolved in glycerol since it was not soluble in water. The mixture was homogeneously spread over a Teflon plate of 15 cm in diameter and was left to dry in an oven at 37 °C for 48 h. These conditions were established after previous experiments to ensure that homogeneous and continuous films without cracks and/or pinholes were obtained. Control films without Ln extract were also prepared for comparative purposes. The obtained films were removed from the plates and equilibrated for two days in a desiccator at 20 ºC and 53% relative humidity (RH), by using oversaturated solutions of magnesium nitrate-6-hydrate (Panreac Química, SA, Castellar del Vallés, Spain). Film thickness was measured in quintuplicate using a hand-held digital micrometer (Palmer-Comecta, Spain, ±0.001 mm) and the average value was used in mechanical, water vapor and oxygen permeability calculations.

Films' nomenclature was 'Ag' for control agar film, 'Alg' control alginate, 'Ag/Alg' control agar and alginate, 'Ag/Ln' agar film with extract, 'Alg/Ln' alginate film with extract, 'Ag/Alg/Ln' agar and alginate film with extract.

2.8.1. Morphological characterization

The microstructural analysis of the cross-sections of the dried films was carried out using Scanning Electron Microscopy (SEM) (Hitachi S-4800). The films (three samples per formulation) were frozen in liquid nitrogen 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. Samples were gold–palladium coated and observed using an accelerating voltage of 10 kV and a working distance of 10 mm.

2.8.2. Mechanical properties

Tensile properties were determined using a Mecmesin MultiTest universal test machine (Landes Poli Ibérica, S.L., Barcelona, Spain) equipped with a 100-N static load cell, according to ASTM standard method D882-09 18 (ASTM, 2010a). Tensile strength (TS), elongation percentage at break (EAB) and elastic modulus (E) were determined from the stress-strain curves. Equilibrated specimens were mounted in the film extension grips and stretched at 50 mm min^{-1} until breaking. Prior to the test, the thickness of the samples was randomly measured at five points. Eight replicates of each formulation were tested.

2.8.3. Physicochemical characterization of the films

2.8.3.1. Water vapor permeability (WVP)

WVP was measured, in triplicate, following the ASTM E96/E96M-10 (ASTM 2010b) gravimetric method for hydrophilic films, using Payne permeability cups of 3.5 cm in diameter (Elcometer SPRL, Hermelle/s Argenteau, Belgium). 5 mL of distilled water was used inside the testing cup to achieve 100% RH on one side of the films, while and

oversaturated magnesium nitrate solution was used to control de RH on the other side of the film: 53% RH. During WVP testing, the side of the films in contact with the Teflon plate was placed in contact with the part of the test having the highest RH. Cups with aluminium samples were used as control samples to estimate solvent loss through the sealing. The water vapor transmission rate (WVTR) was measured at 20 ºC for each type of film. The WVP was determined form WVTR values, as previously described by Fabra, Talens, Gavara, & Chiralt (2012).

2.8.3.2. Oxygen permeability

Permeability to oxygen (OP) was calculated from oxygen transmission rate (OTR) measurements recorded using an Oxygen Permeation Analyzer M8001 (Systech Illinois, UK). Experiments were carried out in triplicate at 23 °C and 80% RH. The samples were previously purged with nitrogen in the humidity equilibrated test cell, before exposure to an oxygen flow of 10 mL min^{-1}. The exposure area during the test was 5 cm^2 for each sample. In order to obtain the oxygen permeability, film thickness and gas partial pressure were considered in each case. Four replicates per formulation were made.

2.8.4. Transparency

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. A spectrocolorimeter CM-3600d (Minolta Co., Tokyo, Japan) with a 10 mm illuminated sample area was used. Internal transmittance (Ti) of the films was quantified as previously reported by Fabra, Talens, & Chiralt (2009). Moreover, CIE-L* a* b* coordinates were obtained from the reflectance of an infinitely thick layer of the material. Measurements were taken in triplicate for each sample.

2.8.5. *In vitro* release assays

The release of phenolic compounds from the films containing extract (Ag/Ln, Alg/Ln, Ag/Alg/Ln) was assessed in two different food simulants. Concretely, 50% ethanol and 10% ethanol were selected as food simulants, according to the Commission Regulation 10/2011 EU on plastic materials and articles intended to be exposed to food (10/2011/EC). For this analysis, the films (pieces of 2 x 2 cm) were immersed in 10 mL of the release medium during different times. Aliquots (0.20 mL) of the supernatant were taken for samples analysis and the released total phenolic compounds (TPC) content was determined in each time interval as described by Singleton, Orthofer, & Lamuela-Raventos (1999) using Folin-Ciocalteau reagent. The aliquot volume was then replaced by fresh release medium. The eluted from the control films without extract (Ag, Alg, Ag/Alg) were considered. The relative percentage was calculated considering the phenolic compounds content of Ln extract used for the film preparation as 100%. Experiments were performed at room temperature (20 °C) in independent triplicates.

2.8.5.1. Antioxidant activity

The antioxidant capacity of active films was evaluated through the Trolox equivalent antioxidant capacity (TEAC) according to previously described in 2.7 section using 17 µL of eluted extracts from films. The TEAC of the film samples was determined by comparing the corresponding percentage of absorbance reduction at 6 min with the Trolox concentration–response curve. All the determinations were carried out in triplicate using a CLARIO star (BMG LABTECH) spectrophotometer, using 50 or 10% ethanol solutions as blanks.

2.8.6. Antimicrobial activity

The antilisterial activity of the films containing Ln extract was tested against *L. innocua*. 8,5 cm of each film were cut and placed in a glass bottle containing 10 mL of MHB. 100 µL of the microorganism in exponential phase were transferred to the samples and

incubated at 37 ºC for 24 h. Control tubes without films and with films without extracts were prepared. Depending on the turbidity of the tubes, serial dilutions with peptone water were made and plated in Petri dishes of selective Palcam agar. Colonies were counted after incubation at 37 ºC for 24 h. The experiment was carried out in triplicate.

2.9. Challenge tests

Locally purchased blueberries were exposed to UV for 15 min in a laminar flow hood to reduce the microbial load. MNV suspension (about ca. 5-6 log $TCID_{50}$/mL) were seeded by distributing 50 µL on the surfaces of fresh blueberries. Inoculated samples were air dried in a laminar flow hood for 60 min. Thereafter each blueberry was immersed for 2 min into Ln coatings and let them dry for 20 min. Finally, samples were stored at 10 and 25 °C. On each sampling day, individual blueberry was placed in a tube containing 5 mL of DMEM supplemented with 10% FCS and shaken for 2 min at 180 rpm to release viral particles from the surface. Blueberries were removed from the DMEM suspension, and then viruses were recovered and titrated as described above. Each treatment was performed in triplicate. Positive controls were uncoated blueberries and coated berries without Ln extract in its formulation under the same experimental conditions. The decay of MNV titers was calculated as described above.

2.10. Statistical analysis

Each experimental value is expressed as the mean ± standard deviation (SD). The statistical analysis of experimental data was performed using InfoStat software (Student Version, 2011). The one-way ANOVA with Tukey post-test at a confidence level of 95% was used to evaluate the significance of differences between groups. The criterion of statistical significance was taken as $p \leq 0.05$.

3. Results and discussion

3.1. Preparation and characterization of phenolic enriched extracts of medicinal plants

Polyphenolic-enriched extracts are of considerable interest and have received increased attention in recent years due to their bioactive properties. In the first part of this work, the antioxidant and antimicrobial (antiviral and antibacterial) activity against foodborne pathogens (*L. innocua*, *E. coli* and MNV) of the five hydroalcoholic extracts, having a content of TPC between 354.7 to 397.9 mg GAE/g dry extract (Moreno et al., 2018 a, b,c) were analyzed.

The antioxidant activity expressed as mmol Trolox/g extract of the different extracts, is gathered in Table 1. As observed, all extracts displayed similar antioxidant activity which ranged between 2.7 and 4.9 mmol Trolox/g extract, being significantly higher ($p<0.05$) for Zp, Ln and Lc extracts. It should be noted that the antioxidant activity of the five species was higher than that reported for extracts of other *Larrea* plant species (Aguirre-Joya et al., 2018; Varela, Arslan, Reginato, Cenzano, & Luna, 2016). However, higher antioxidant properties were previously reported for other natural extracts such as green tea extracts (GTE) and grape seed extract (GSE) (Magcwebeba et al., 2016; Majchrzak, Mitter, & Elmadfa, 2004).

Table 1. Antioxidant capacity (TEAC: Trolox equivalent antioxidant capacity) of medicinal plant extracts.

Sample	TEAC (mmol Trolox/g extract)
Tc	3.0 (0.1)[a]
Zp	4.9 (0.3)[b]
Ln	4.5 (0.1)[b,c]
Ld	2.7 (0.2)[a]

Lc	3.9 (0.3)^{c}	

Zp: *Zuccagnia punctata;* Ld: *Larrea divaricata*; Ln: *Larrea nitida;* Lc: *Larrea cuneifolia;* Ta: *Tetraglochin andina*. Different superscripts indicate significant differences among extracts ($p < 0.05$). Mean values (standard deviation).

The microbiological risks of food products even today are one of the main sources of foodborne diseases, being human enteric viruses the most common etiologic agents identified in foodborne outbreaks (Bennett et al., 2018). Furthermore, *Listeria* is another important foodborne pathogen since listeriosis can cause serious problems in newborns, pregnant women, the elderly and immunocompromised individuals, with a high mortality rate of 30% that exceeds that of other common diseases transmitted by food (Realini & Marcos, 2014).

In this work, both *Listeria innocua* and *Escherichia coli* were chosen due to their relevance in foodborne illnesses. The evaluation of antibacterial activity of the five plant extracts against both microorganisms was quantitatively assessed by determining MIC and MBC values and the results are shown in Table 2. Results clearly demonstrated that the antibacterial activity was higher for *L. innocua* than *E. coli* which can be attributed to the differences in the cell wall structure. This can be related to the fact that Gram-negative microorganisms are less susceptible to the action of the antibacterial compounds since they possess an outer membrane surrounding the cell wall that limit diffusion of hydrophobic compounds through its lipopolysaccharide covering (Harvey, Champe, Fisher, & Strohl, 2007).

As observed, Ln presented stronger antilisterial activity than the other evaluated plant extracts, with MIC and MBC values of 100 and 350 µg/mL, respectively. As compared with literature, Ln extract showed greater or similar antibacterial efficacy than other natural extracts. Cosentino et al. (1999) showed MBC values of commercial thymus oil against *L. monocytogenes* of 0.9 mg/mL, while hydroalcoholic murta extracts presented MBC values between 0.1 and 2.2 mg/mL (López de Dicastillo et al., 2017) and

Cinnamomum javanicum plant extract had a similar MIC value but higher MBC (0.13 mg/mL and > 2 mg/mL, respectively) (Yuan, Wen, & Yuk, 2017).

Table 2. Antibacterial activity of Argentinian plant extracts against *L. innocua* and *E. coli*.

Sample	*L. innocua*		*E. coli*	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
Zp	300	>800	>800	>1000
Ld	200	550	800	>1000
Ln	100	350	800	>1000
Lc	300	550	800	>1000
Ta	>500	>700	>1000	>1000

Zp: *Zuccagnia punctata;* Ld: *Larrea divaricata*; Ln: *Larrea nitida;* Lc: *Larrea cuneifolia;* Ta: *Tetraglochin andina*. MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration.

Moreover, human enteric viruses account for the major causes of foodborne outbreaks in high-income countries and, thus, research interest has recently increased in the identification of natural compounds (i.e. essential oils and polyphenols enriched plant extracts) with antiviral activity (Randazzo, Fabra, Falcó, López-Rubio, & Sánchez, 2018; Abdelkebir et al., 2019). Figure 1 summarizes the antiviral activity of the evaluated plant extracts against MNV measured at 37 ºC where it is clearly evidenced that Ln and Ld were the most effective extracts in reducing the titers of MNV. Incubation of MNV with both plant extracts at concentrations of 0.5 and 5 mg/mL reduced MNV titers to undetectable levels after ON incubation at 37 °C. MNV titers were reduced by 1.9, 2.5, and 2.6 log $TCID_{50}$/mL, after treatment with 5 mg/mL of Ta, Zp, and Lc extracts, respectively.

Previously, the dry extracts used in this study were chemically characterized and two quercetin derivatives flavonoids and 10 major lignans were identified. Trihydroxy-6,7´cyclolignan was found only in Ld extract and dihydroxy-methoxy-epoxylignan in Ln extract, and nordihydroguaiaretic acid (NDGA) was found in extracts obtained from both plant species (Moreno et al., 2018b). Consequently, the high antimicrobial activity of Ln and Ld extracts can be attributed, in part, to the presence of quercetin derivatives and lignans principally nordihydroguaiaretic acid (NDGA). In fact, the antiviral activity of similar polyphenolic extracts with high concentrations of quercetin has been recently demonstrated (Falcó et al., 2018) and the antiviral activity of NDGA and its derivatives have been previously reported against human immunodeficiency virus, human papilloma virus, herpes simplex virus, influenza virus and Junin virus (Palacio, Cantero, Cusidó, & Goleniowski, 2012).

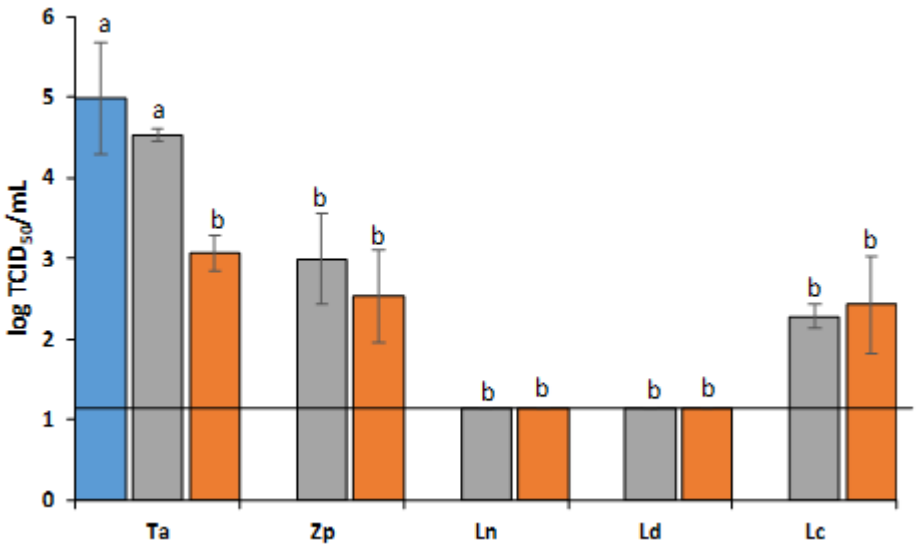


Figure 1. Effect of Argentinian plant extracts on MNV infectivity. Blue bar indicates untreated samples, greys bars indicate 0.5 mg/mL plant extract and orange bars 5 mg/mL plant extract. Solid line indicates the detection limit for the $TCID_{50}$/mL assay. Titers are the means ± standard deviations of results of three replicates.

401 Therefore, considering that Ln extract showed the highest antimicrobial activity for both
402 *L. innocua* and MNV, and high antioxidant activity, this extract was chosen for the
403 development of active edible coatings. To this end, the toxicity of the Ln extract by means
404 of Ames and *A. salina* was evaluated in order to evaluate its applicability in food-related
405 products.
406 Results obtained from the *A. salina* test showed that the Ln extract was not toxic below
407 the concentration of 500 μg/mL. The LC_{50} value was of 750 μg/mL, while potassium
408 dichromate used as a positive control exhibited a LC_{50} of 30 μg/mL. Table 3 displays the
409 results obtained in the mutagenic activity evaluation assay against S. *typhimurium* TA98
410 and TA100 strains. As observed, the mutagenicity test using strains of *S. typhimurium*
411 TA98 and TA100 indicated that, up to a concentration of 700 μg/plate, the Ln extract did
412 not induce an increase in the number of spontaneous revertants, showing in all cases MR
413 <1.5. At the concentration of 1400 μg/plate, the effect against either of both strains could
414 not be determined, given that bacterial viability is affected at that concentration, given
415 the antimicrobial activity of the extract against *S. Typhymurium*.
416
417 **Table 3**. Results obtained in the mutagenic activity evaluation assay against *S.*
418 *Typhimurium* TA98 and TA100 strains.

Sample	**μg GAE/ plate**	**TA98 (-) S9**	**T100 (-) S9**	**MR TA 98**	**MR TA 100**
Ln extract	1400	ND	ND	ND	ND
	700	38 (1)	122 (21)	1.11	1.10
	350	36 (1)	117 (9)	1.05	1.06
Positive control[1]		1222 (109)	723 (171)		
Negative control[2]		34 (2)	110 (16)		

* MR: mutagenicity relation; ND: not determined. These concentrations affect the viability of the *Salmonella* strains. [1] mean number of revertants induced by 4-nitro-o-phenylendiamine (10 μg/plate). [2] The number of spontaneous revertant colonies determined without the addition of the samples, only with the vehicle, DMSO. Mean value (standard deviation).

Thus, it can be concluded that Ln extract is not toxic or genotoxic at the concentrations tested which would guarantee its safe use and allow its incorporation into active food coatings. Therefore, Ln extract was selected as active compound for the development of antiviral, antibacterial and antioxidant edible coatings for food applications. The physicochemical, antioxidant and antimicrobial properties of the developed active films were characterized, and the antiviral efficiency on blueberries coated with polysaccharide-based coatings containing Ln was carried out.

3.2. Properties of stand-alone polysaccharide-based films

Three different polysaccharide-based matrices (agar, alginate and agar/alginate mixtures) were used as biopolymer coatings in which the Ln extract was incorporated to confer them active properties. Morphological, physicochemical and functional properties of edible coatings, including mechanical, water vapor/oxygen barrier and optical properties were characterized.

Figure 2 shows representative SEM micrographs of the cross-section images of the developed polysaccharide-based films after a one-week storage period at 25 ºC and 53% RH. Neat alginate or agar films exhibited a quite homogeneous structure with no brittle areas or bubbles, consistent with the formation of a compact arrangement of polymer chains. Both polysaccharides are highly compatible as deduced from the SEM images since no phase separation was observed. Therefore, it can be postulated that interactions between film components (alginate, agar and glycerol) favored the integration of polysaccharides in a homogeneous matrix. In contrast, incorporation of the Ln extract provided a slightly rougher structure, being more accentuated in films prepared with a mixture of both polysaccharides.

Figure 3 shows typical Ti spectra distribution curves of films, from 400 to 700 nm, as a transparency indicator, which is directly linked with the internal structure of the developed films (Fabra et al., 2009). An increase in the internal distribution of transmittance is ascribed to an increase in transparency. From the films tested, those prepared with pure phyco-colloids were the most transparent, regardless the type of polysaccharide used. In contrast, the presence of the Ln extract significantly reduced the transparency of polysaccharide based films, showing lower Ti values in all the wavelength considered, indicating that these films were more opaque and heterogeneous than their counterparts prepared with pure alginate and/or agar. This can be ascribed not only to the fact that transmittance of the samples is affected by differences in refractive indices between the neat polysaccharide matrix and the different compounds present in the Ln extract, but also by the light selective absorption of polyphenol compounds of Ln extract at low wavelengths. In fact, although all tested films preserved good contact transparency (see Figure 4), it was slightly diminished when Ln extract was incorporated in the biopolymer matrices since these polyphenol compounds impart a yellowish color to the films, thus decreasing the hue and Ti values at low wavelengths. Plant extracts commonly provide opacity to polymers and, as a result, films containing extracts are less transparent than films without extracts (Norajit, Kim, & Ryu, 2010). The addition of plant extracts in films provides an adequate barrier to light, which is important to prevent the degradation of light sensitive components (Mir et al., 2018). In fact, previous works have reported a greater protection against UV light in biopolymer matrices containing plant extracts as compared to their counterparts prepared without extracts (Abdollahi, Damirchi, Shafafi, Rezaei, & Ariaii, 2019; Fabra, Falcó, Randazzo, Sánchez, & López-Rubio, 2018; Mir et al., 2018).

From the reflectance spectra of an infinite thickness film, Lightness (L*), hue (h^*_{ab}) and Chroma (C^*_{ab}) were obtained as well as the total color differences (ΔE) with respect to the neat polysaccharide films (Table 4). Incorporation of Ln extract provided darker (lower L*) films, with a more saturated (higher C_{ab}*) orange-brownish color (lower h_{ab}*).

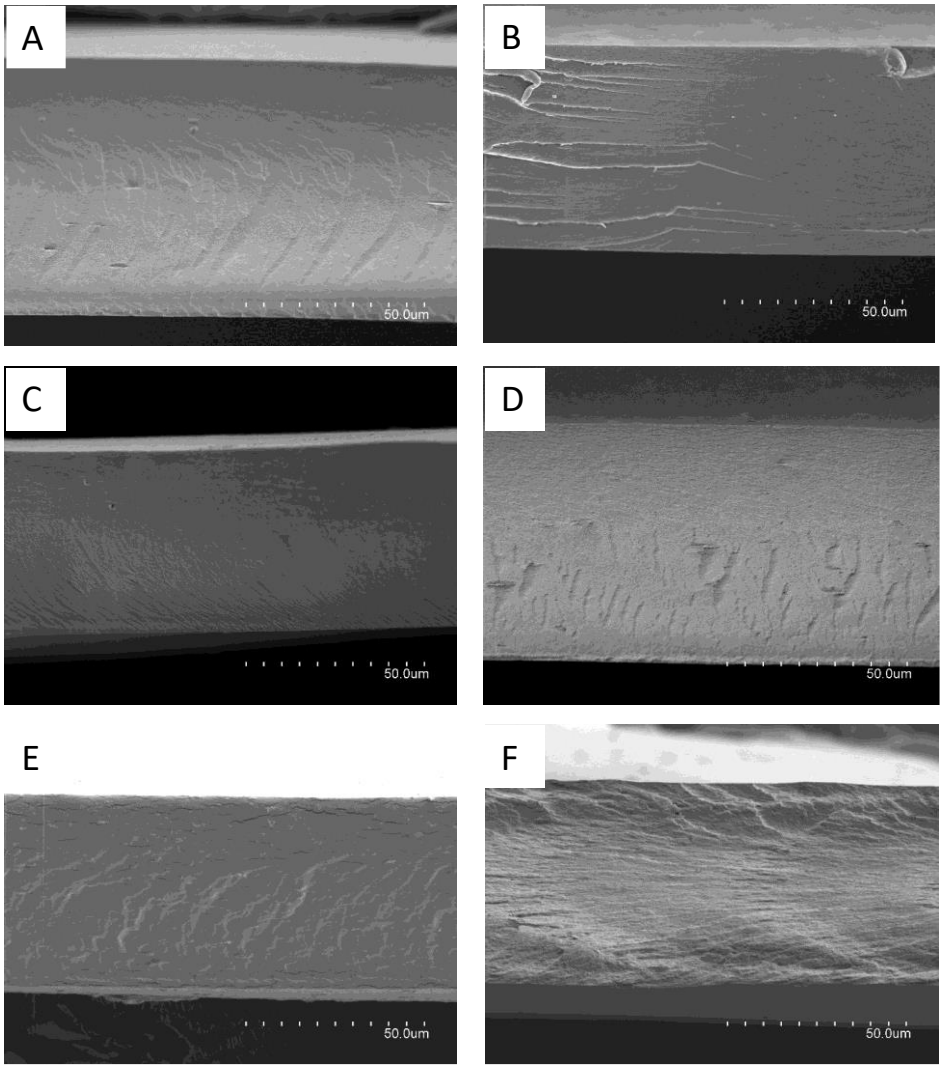
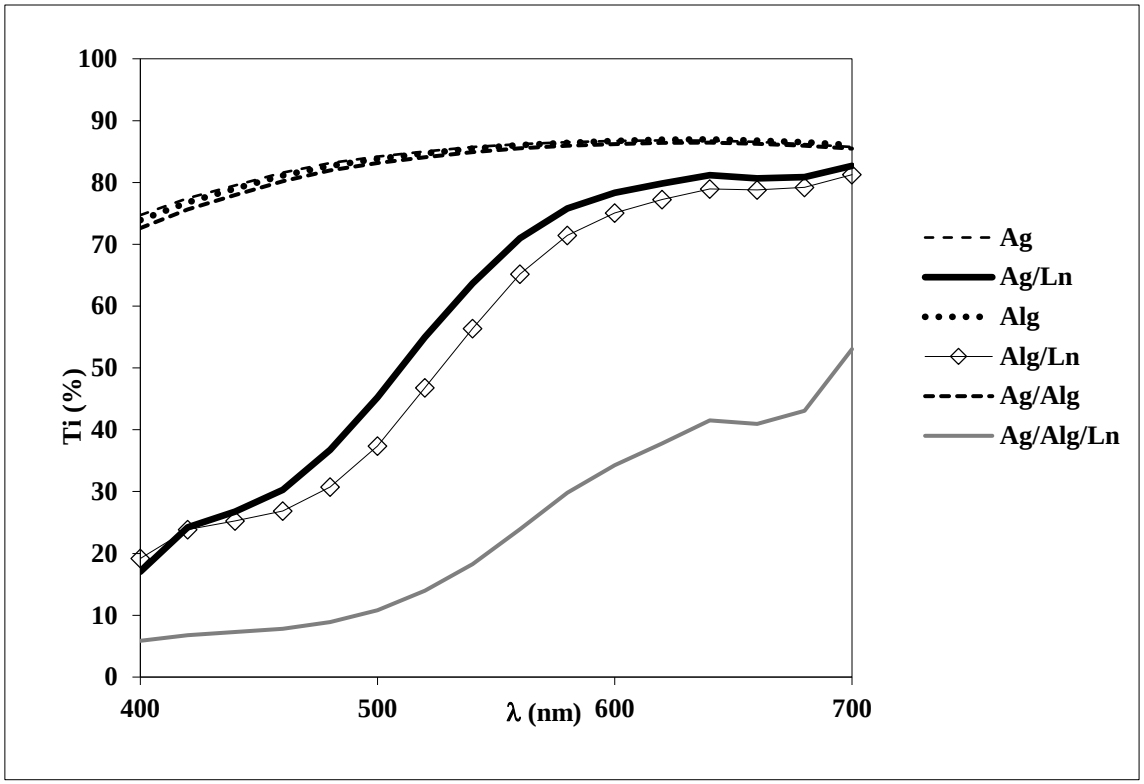
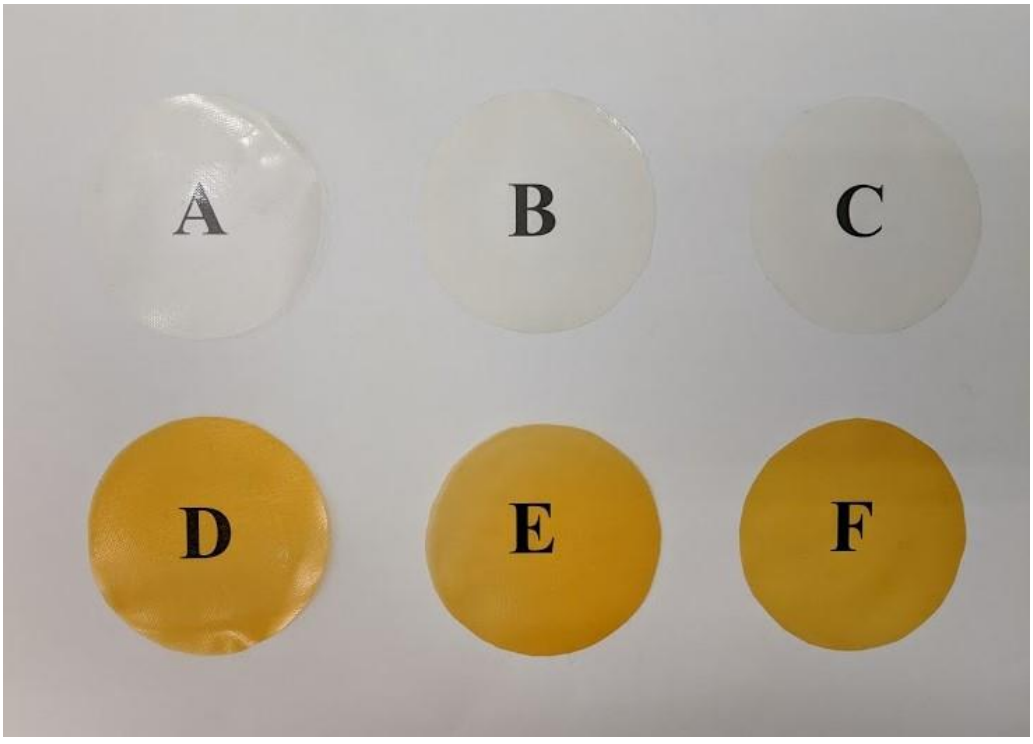


Figure 2. Cross-section images of the developed films. **A**: control agar film; **B**: agar with Ln extract; **C**: control alginate; **D**: alginate with Ln extract; **E:** control agar/alginate; **F**: agar/alginate with Ln extract.



482

483 **Figure 3.** Spectral distribution of internal transmittance (Ti) of the developed stand-alone films.



484

485 **Figure 4.** Contact transparency pictures of the developed stand-alone films. **A**: agar control; **B**:
486 alginate control; **C**: agar/alginate control; **D**: agar/Ln; **E**: alginate/Ln; **F**: agar/alginate/Ln.

487

488 **Table 4.** Color parameters of the developed stand-alone films.

Films	L*	C^*_{ab}	h^*_{ab}
Ag	79.9 (0.3)	20.4 (0.5)	85 (1)
Ag/Ln	52.0 (1.0)	40.2 (0.3)	62 (2)
Alg	78.4 (0.3)	21.7 (0.5)	81 (3)
Alg/Ln	48.1 (0.9)	36.5 (0.5)	57 (1)
Ag/Alg	76.7 (1.5)	20.3 (1.0)	83 (2)
Ag/Alg/Ln	54.4 (1.0)	40.1 (1.5)	63 (2)

489 L* lightness, C^*_{ab} Chroma, h^*_{ab} hue. Different superscripts within a column indicate significant
490 differences among formulations ($p < 0.05$). Mean values (standard deviation).
491

492 Tensile properties are useful parameters with which to describe the mechanical behavior
493 of films and are closely related with its internal structure. Table 5 displays the tensile
494 parameters (elastic modulus (E), tensile strength (TS), and elongation at break (EAB)) of
495 the tested films prepared with and without Ln extract. Neat polysaccharide films were
496 highly resistant and stiff with values in the range of those previously reported in the
497 literature (Barbut & Harper, 2019; Kanmani & Rhim, 2014; Shankar & Rhim, 2017),
498 being agar-based films stiffer (higher E values) than alginate or agar/alginate films. It is
499 observed that the incorporation of Ln extract did not significantly modify the
500 stretchability and stiffness of the films, although a tendency to decrease the mean TS
501 values was observed for Ln-containing edible coatings, suggesting that the Ln extract had
502 a weakening effect on the films, hence showing the interruption effect of the extract in
503 the polysaccharide network which is what provides most of the tensile strength. Similar
504 effects have been reported in protein and polysaccharide-based films incorporating
505 hydrophobic compounds (Fabra et al., 2008; Khwaldia, Banon, Desobry, & Hardy, 2004).

The small differences observed in the present work could be explained by the low amount of Ln added into the polysaccharide matrices.

Table 5. Mechanical properties[1] of the stand-alone coatings.

Sample	TS (MPa)	E (MPa)	EAB (%)
Ag	27.5 (6.8)^{a}	992 (52)^{a}	19.7 (7.1)^{a}
Ag/Ln	19.5 (2.6)^{a,b}	970 (30)^{a}	14.5 (3.5)^{a}
Alg	22.4 (9.1)^{a,b}	793 (94)^{b}	17.8 (10.9)^{a}
Alg/Ln	10.3 (2.1)^{b}	784 (24)^{b}	10.6 (3.2)^{a}
Ag/Alg	24.0 (8.3)^{a,b}	615 (41)^{c}	23.7 (4.3)^{a}
Ag/Alg/Ln	12.9 (3.9)^{a,b}	477 (42)^{c}	21.4 (5.1)^{a}

[1] Elastic modulus (E), tensile strength (TS), and elongation at break (EAB) of films equilibrated at 53% relative humidity for one week. Different superscripts within a column indicate significant differences among formulations ($p < 0.05$). Mean values (standard deviation).

Barrier properties, usually described by their permeability values, are important because they will affect oxidation and respiration rates in the enclosed foods and thus, they are the most important factors to evaluate the effectiveness of edible coatings. Table 6 gathers water vapor and oxygen barrier properties data of developed edible films. WVP values ranged between 7.8 and 8.5 g Pa^{-1} $s^{-1}m^{-2}$ for neat polysaccharide matrices, in agreement with those found in literature for agar-based films (Roy, Rhim, & Jaiswal, 2019) although they were slightly lower than those previously reported for alginate-based films (Rhim, 2004; Wang, Shankar, & Rhim, 2017). Interestingly, the incorporation of Ln extract significantly reduced the WVP probably due the higher hydrophobic nature of the extract (which was not soluble in water).

Oxygen permeability (OP) was measured at 53% RH and the results are also summarized in Table 6. The OP values for the neat alginate and agar-based films agree with that

reported in the literature (Rojas-Graü et al., 2007, Zhang et al., 2019). The high permeability values obtained can be related to the fact at, at high RH, hydrophilic films tend to loss their gas barrier properties due to the increase of polymer chain mobility (Forssell, Lahtinen, Lahelin, & Myllärinen, 2002). It is noticeable that neat agar-based films were much more permeable to oxygen than their counterparts prepared with alginate (either Alg or Ag/Alg). Curiously, the incorporation of Ln extract lead to an increase in the OP values, except in the case of agar matrices where it was significantly decreased by approximately 46.45%, showing no significant differences ($p<0.05$) with their counterparts prepared with alginate and Ln. Thus, similar OP values were observed for Ln-containing edible films.

The different behavior of Ln extract when it was incorporated into polysaccharide matrices can be explained by changes in the film microstructure, thickness, void volume in the biopolymer structure and different arrangement of the biopolymer chain. In fact, there is a controversy in the literature concerning the barrier properties behavior, based on the type of hydrocolloid and the nature of plant extract. For instance, Nouri & Mohammadi Nafchi (2014) also reported an increase in OP values due to the incorporation of betel leaves extract in starch-based films. In contrast, Akhtar et al. (2013) reported a decrease in OP values of HPMC films when a commercial plant extract (betacyanins) were added. They attributed this improvement in the OP barrier properties to availability of free hydroxyl groups of natural extract to interact with the HPMC matrix by hydrogen bounds, giving a more compact structure of polymer matrix. On the other hand, Ekrami, Emam-Djomeh, Ghoreishy, Najari, & Shakoury (2019) did not find significant differences of OP values of Salep mucilage films with or without different amounts of pennyroyal extracts.

Table 6. Water vapor and oxygen permeability of the developed stand-alone films.

Sample	WVP 10^{-10} (g Pa^{-1} $s^{-1}m^{-2}$)	OP 10^{-20} (m^3 m m^{-2} s^{-1} Pa^{-1})
Ag	8.47 (0.27)[a]	13.95 (3.69)[a]
Ag/Ln	6.10 (1.77)[b]	7.47 (1.48)[b]
Alg	8.30 (0.52)[a]	1.77 (0.79)[c]
Alg/Ln	6.04 (0.10)[b]	4.16 (0.03) [b,c]
Ag/Alg	7.83 (0.19)[a,b]	1.89 (0.27)[c]
Ag/Alg/Ln	6.06 (0.17)[b]	3.76 (1.45) [b,c]

Different superscripts within a column indicate significant differences among formulations ($p < 0.05$). Mean values (standard deviation).

The antibacterial activity of pure polysaccharide based films and those containing Ln extract is shown in Table 7. The first thing to highlight is that even though the reduction was not complete, an inhibitory effect was observed for all films containing 500 μg/mL Ln extract. In fact, the incorporation of Ln extract led to a significant reduction ($p<0.05$) in the growth of *L. innocua*, thus showing a certain degree of antibacterial activity. The higher reduction found in the alginate and alginate/agar films containing Ln extract can be related to the fact that alginate is highly hydrophilic, and it mostly disintegrated in the aqueous culture medium (MHB).

566 **Table 7.** Antimicrobial effectiveness against *L. innocua*. Ln films at 37 °C expressed as
567 logarithm of colony forming units per milliliter (Log CFU/mL) and log reduction value
568 (LRV).

Sample	Log CFU/mL	LRV
Control without film	8.12[b]	
Ag	8.18 (0.04)[b]	
Ag/Ln	5.21 (0.72)[a]	2.97
Alg	8.17 (0.17)[b]	
Alg/Ln	5.57 (0.68)[a]	2.60
Ag/Alg	8.36 (0.07)[b]	
Ag/Alg/Ln	5.36 (0.28)[a]	3.00

569 Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).
570 Mean values (standard deviation).

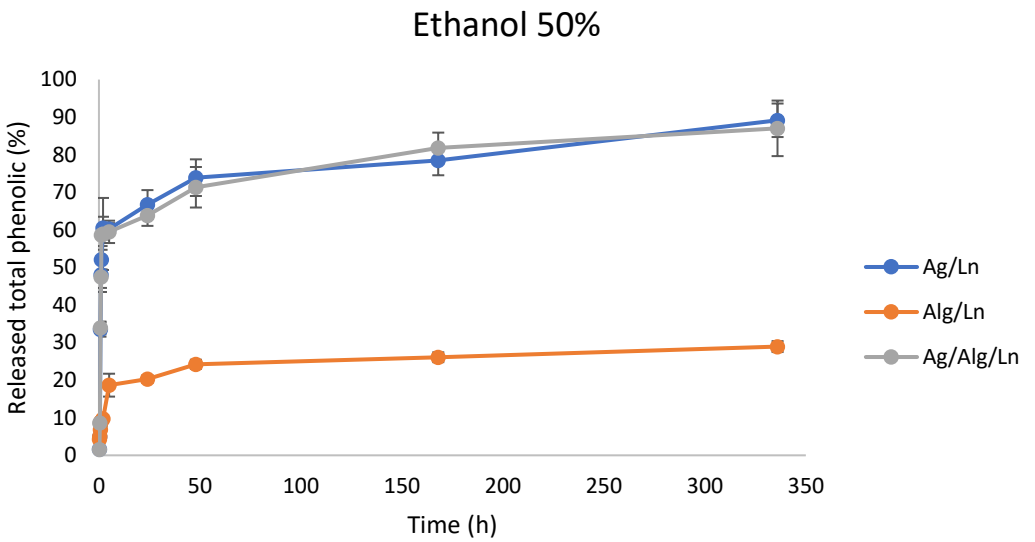
571

572 To estimate the amount of released extract after the antibacterial test, the content of
573 released phenolic compounds in the medium and the amount remaining in the film were
574 quantified. Results showed that only a 34.78 ± 3.52% of Ln extract was released to the
575 assay medium whereas the polyphenol content remaining in the film was around 61.01 ±
576 3.87%, fact that could also explain that the films did not show the expected bacterial count
577 reduction against *L. innocua* since the amount released after the antibacterial test was
578 lower than the obtained MBC for Ln (350 μg/mL, Table 2).

579 The release of Ln extract from the developed polysaccharide based films was assessed in
580 two different media, by determining the released TPC content, and the results are shown
581 in Figure 5. Ethanol 10% and 50% (v/v) were selected as food simulants with different
582 hydrophilicity, according to the Commission Regulation 10/2011 EU (10/2011/CE)
583 (Commission, 2011).

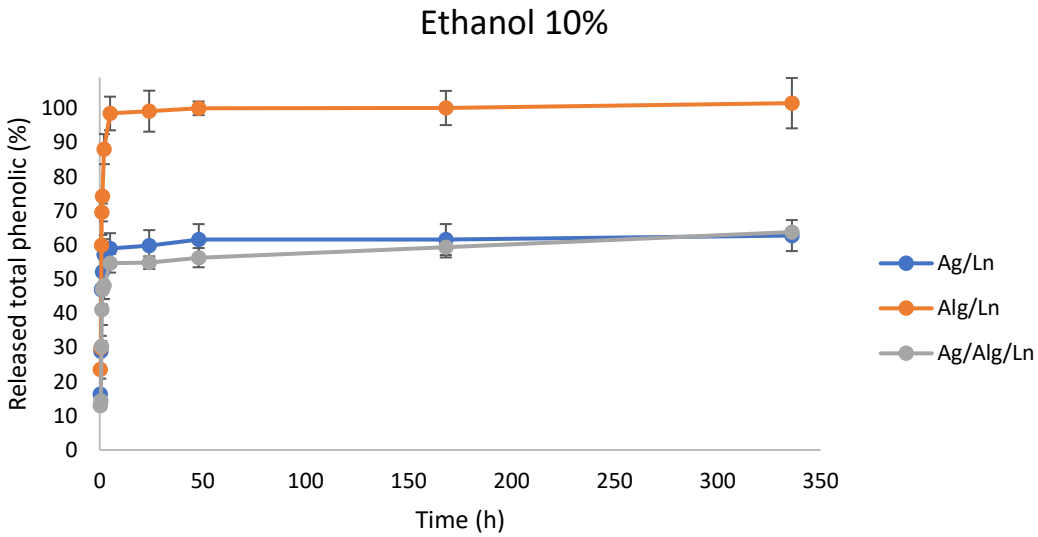
An initial burst release was observed in both food simulants, which is typical of hydrophilic biopolymer matrices that rapidly swell in hydroalcoholic media (Moreno et al., 2018c). Interestingly, differences in the TPC from Ln released were observed depending on the food simulant used, which is linked with the solubility and disintegration of the polysaccharide matrices in the media. In fact, when 50% ethanol was used as a food simulant, the Ag/Ln and Ag/Alg/Ln films behaved similarly, releasing 89.2 ± 4.4 and 87.0 ± 7.4% of TPC, respectively, while the alginate films only released 28.9 ± 1.4% of TPC after 14 days in this medium. In contrast, when 10% ethanol was used; the Ag/Ln and Ag/Alg/Ln films also behaved similarly but only a 62.7 ± 6.2 and 63.8 ± 0.2% were released, respectively, after 14 days whereas, the Alg/Ln film was completely dissolved after 5 h in contact with the medium, achieving 100% of TPC released. In all cases, the developed Ag/Ln and Ag/Alg/Ln could release significant amounts of TPC from the Ln extract in the two different food simulants (being higher in 50% ethanol where the Ln extract was better solubilized) whereas small amount of TPC (~30%) was released in 50% ethanol when it was incorporated into pure alginate (Alg) matrices. Similarly, Ruan et al., 2019 reported a maximum release of 30% epigallocatechin-gallate from sodium alginate-carboxymethyl cellulose edible films immersed in 95% ethanol, which was associated with the interaction between EGCG and the biopolymer matrix.

603 A



604

605 B



606 **Figure 5.** Release of TPC from the films in 50% ethanol (A) and 10% ethanol (B).

607 In order to further confirm these results, the antioxidant activity of the Ln extract released
608 to the simulant media was quantified after 14 days by the ABTS assay for all the
609 polysaccharide matrices. The results were compared with the antioxidant activity of pure
610 Ln extract, and are compiled in Table 8. Data are expressed as mmol Trolox/g Ln extract
611 (TEAC values), by considering the Ln mass fraction in the dry sample. The estimated
612 mmol Trolox/g extract, obtained from the antioxidant activity of pure Ln extract and the

amount of Ln release in each food simulant (Figure 5), was also calculated for comparative purposes.

Interestingly, the amount of TPC released and the antioxidant capacity were well-correlated. In general, comparable antioxidant activity of Ln extract in both media was observed for both samples Ag/Ln and Ag/Alg/Ln, thus indicating that the presence of agar in the biopolymer matrices played an important role on the release. It is worth noting that the estimated mmol Trolox/g extract was slightly lower than the experimental values obtained in 50% ethanol whereas the opposite effect was observed in 10% ethanol, thus suggesting that the TPC released in both simulant media were not exactly the same, having a higher antioxidant capacity those with lower polar character (released in 50% ethanol).

It should be noted that the antioxidant activity was similar (~ 4.1 -4.3 mmol Trolox/g extract) for the ~90 % Ln extract released from Ag/Ln and Ag/Alg/Ln edible films in a food simulant with 50% ethanol than for the ~100 % Ln extract released from pure alginate films in 10% ethanol (where the film was completely disintegrated). In addition, no significant differences were observed between the maximum antioxidant activities of Ln released compared to the pure Ln extract, thus, suggesting that the Ln extract was not degraded during film formation.

In contrast, the antioxidant activity of the alginate films in the 50% ethanol medium was only 2.1 ± 0.2 mmol Trolox/g extract, whereas up to 4.1 ± 0.3 mmol Trolox/g extract was the antioxidant activity value reached for the alginate films immersed in 10% ethanol medium, which was in the same range of the antioxidant activity of pure Ln (4.5 ± 0. mmol Trolox/g extract).

637 **Table 8.** Antioxidant activity (TEAC: Trolox equivalent antioxidant capacity) of the
638 developed active films.

		mmol Trolox/g extract	Estimated mmol Trolox/g extract (*)
	Ln extract	4.5 (0.1)[a]	-
Ethanol 50%	Ag/Ln	4.3 (0.4)[a]	4.0
	Alg/Ln	2.1 (0.2)[b]	1.9
	Ag/Alg/Ln	4.1 (0.2)[a]	3.9
Ethanol 10%	Ag/Ln	2.4 (0.2)[b]	2.8
	Alg/Ln	4.1 (0.3)[a]	4.5
	Ag/Alg/Ln	2.3 (0.2)[b]	2.8

639 Mean value (standard deviation). Different letters in the same column indicated significant differences
640 according to Tukey's test ($p \leq 0.05$).

641 (*) values obtained taking into account the antioxidant activity of the pure Ln extract and the amount of Ln extract released from both
642 food simulants.

643

644 **3.3. Challenge tests**

645 Ln extract with demonstrated antiviral activity has been postulated as potential candidate
646 to develop antiviral coatings. In order to broaden the use of natural compounds for the
647 development of antiviral coatings, agar and alginate-based coatings, with and without Ln
648 extract, were used to coat fresh blueberries artificially inoculated with MNV and stored
649 at 10 °C (ON and 4 days) and 25 °C (ON). Neat agar coatings (without Ln extract) reduced
650 the infectivity of MNV below the limit of detection after ON storage at 25 °C while MNV
651 titers were reduced by 2.54 and 2.88 log after ON and 4 days at 10 °C storage (Figure 6A)
652 since viruses usually persist better at lower temperatures than at higher temperatures.

These results are not entirely surprising since other biopolymeric matrices such as carrageenan also exert antiviral activity on coatings application (Falcó, Randazzo, Sánchez, López-Rubio, & Fabra, 2019). Furthermore, the efficacy of the coatings containing Ln extract was not improved at 25 ºC in agar-based films (Figure 6A), probably due to the intrinsic antiviral activity of the agar. In contrast, the incorporation of Ln in Alg and Alg/Ag edible coatings increased the antiviral activity of the coatings at 10 °C. Alg/Ln edible coatings reduced MNV titers by 1.37, logs, 0.88 and 0.92 logs compared to neat alginate coatings, after ON at 25 °C, ON at 10 °C and 4 days at 10 °C (Figure 6B).

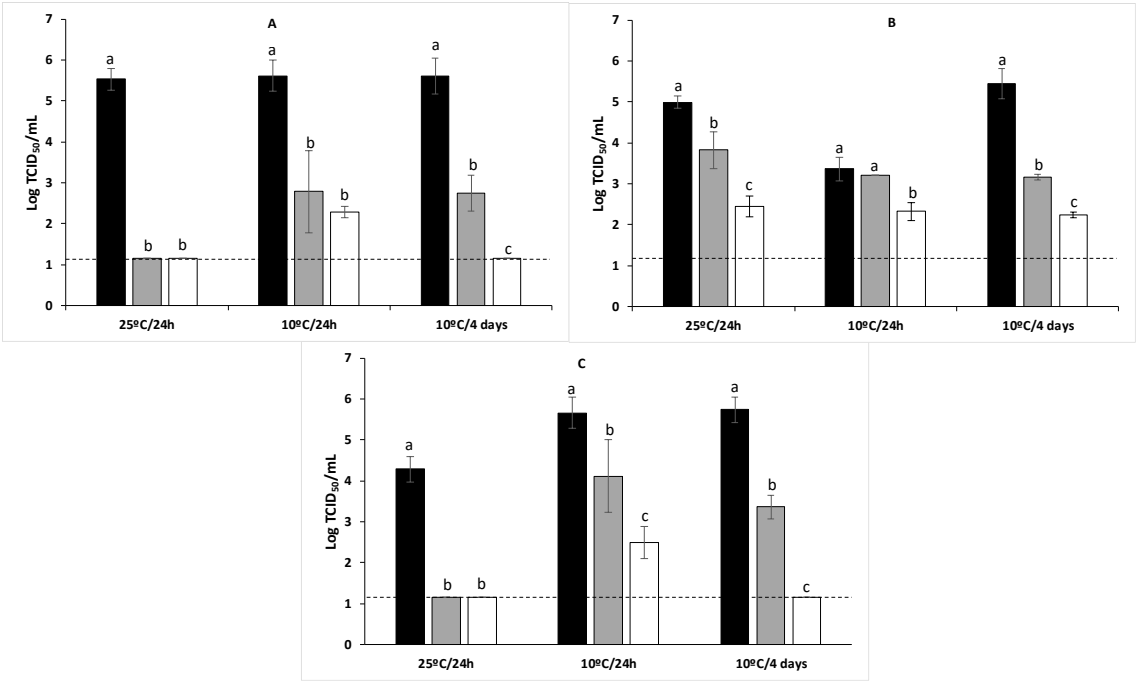


Figure 6. Reduction of MNV titers (log $TCID_{50}$/mL) on blueberries after treatment coatings at different temperatures and storage times. (A: Agar coating; B: Alginate coating; C: Agar/Alginate coating).
*Black bars: virus control; Grey bars: coating control; White bars: coating Ln.
**Dashed lines depict the detection limit.
***Each column represents the average of triplicates. Within each column for each storage condition, different letters denote significant differences between treatments ($P < 0.05$).

4. Conclusions

This work has demonstrated the potential of plant polyphenolic extracts, obtained from plant species that grown in arid and semiarid regions of Argentina, for the development of active edible coatings with potential antimicrobial and antioxidant activities. Different polysaccharide-based matrices (alginate, agar and mixtures of both) were used and the coatings were loaded with Ln extract, which showed the highest antibacterial, antiviral and antioxidant activities. Furthermore, results showed that Ln extract was not toxic or genotoxic at the concentration used for the development of active coating (500 μg/mL) which can ensure its safe used in food-related products.

Regarding the antibacterial activity of the developed stand-alone films, a significant reduction ($p<0.05$) in the growth of *L. innocua* was observed in all films containing 500 μg/mL of Ln extract. The developed active coatings could release significant amounts of Ln extract in two different food simulants. This release was dependent on the polysaccharide matrices, being higher for agar-containing films when they were immersed in a food simulant with a higher ethanol concentration. A faster release was observed for pure alginate-based coatings when they were immersed in 10% (v/v) ethanol solution since the film was greater hydrated and disintegrated.

These active edible coatings containing Ln, which exhibited antioxidant and antimicrobial activity, could be an alternative in the strategies followed to reduce or eliminate human enteric viruses, since they were proved to effectively reduce the titers of MNV in artificially contaminated blueberries.

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