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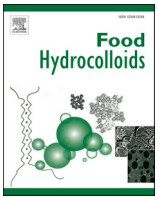

Sustainable bioactive pectin-based films to improve fruit safety via a circular economy approach

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ABSTRACT

This work reports on the valorisation of persimmon (*Diospyros kaki Thunb.*) for the development of food-grade antiviral coatings against major viral foodborne pathogens, human noroviruses (NoVs) and hepatitis A virus (HAV). Initially, the antiviral activity of polyphenol-rich pectin extracts with abundant non-covalent interactions (PPN), pectin extracts enriched with intact pectin-polyphenol ester and O-glycosyl bonds (PPC) and hydro-ethanolic polyphenol-rich extracts (EPE) was compared. Higher viral reductions were found for the pectin extracts rich in polyphenols, mainly in those containing covalent pectin-polyphenol interactions. This specific extract was mixed with commercial citrus pectin (CP) to develop active edible films. Dry films were analysed in terms of their optical, morphological, mechanical and barrier properties. Addition of the bioactive pectin persimmon extract resulted in more coloured films with lower transparency. The presence of covalently-linked polyphenols gave rise to stiffer films, with lower sorption capacity and more hydrophobic nature. The infectivity of MNV and HAV on fresh blueberries after the coating treatments was reduced by approximately 4.28 and 2.38 log, respectively, after overnight incubation, as compared to the controls, when 10% PPC was incorporated into the film. Higher amounts of PPC did not significantly improve the antiviral activity and a complete inactivation for both viruses was observed after 4 days of storage at 25 °C. This paper highlights the potential of persimmon discards as a cheap source of food-grade antiviral coatings with improved physicochemical properties as compared to commercial citrus pectin.

1. Introduction

In the last decade, there has been an increased interest in the preservation of both the environment and the health of consumers. Thus, a great deal of emphasis has been placed on the use of natural and biological products and food consumption habits. For instance, bioprotection has emerged as an important natural strategy for sustainable postharvest management of perishable produce. In particular, biopolymer applications to preserve vegetables freshness and quality of fruits (edible coatings) have emerged as a prospective approach. Both polyphenol-rich extracts and biopolymers are currently being explored as important natural preservatives for preventing bacterial and fungal spoilage in fruits following harvest (Cenobio-Galindo et al., 2019; Gaikwad, Singh, & Lee, 2019). In this sense, recent studies have reported that the application of polyphenol-rich extracts (e.g. green tea extract) incorporated within edible coatings is a highly effective strategy for the preservation of berries quality following harvest and offers substantial protection not only against fungal decay but also antiviral activity (Moreno et al., 2020). In this regard, antiviral edible films and coatings are a promising area to explore due to the risk of disease transmission by food contaminated with enteric viruses. In fact, foodborne viral outbreaks are a growing concern for food safety authorities. A wide variety of viruses may be transmitted by food, nevertheless, the most frequently reported viruses are human noroviruses. Presence of human enteric viruses can occur in food which has been directly contaminated with faecal material or contaminated water. The main foodstuffs involved in foodborne infections are mollusc bivalves, vegetables and salads and berries which have been contaminated during their production or along the supply chain by improper handling after their preparation.

An inexpensive source for biopolymer production could be the use of food industry wastes which can be upcycled for extracting biopolymers or bioactive molecules (Awasthi et al., 2021; Esparza, Jiménez-Moreno,

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Bimbela, Ancín-Azpilicueta, & Gandía, 2020; Méndez, Fabra, Falcó, et al., 2021; Méndez, Fabra, Gómez-Mascaraque et al., 2021).

Persimmon (*Diospyros kaki Thunb*) fruits are considered a valuable functional produce. Their nutritional and medicinal benefits have been attributed to their high content in various nutrients and phytochemicals such as carbohydrates (e.g. pectin), vitamins, proanthocyanidins, phenolic acids, tannins, dietary fibre and carotenoids (Bordiga et al., 2018; Conesa, Laguarda, Pedro, & Lucía, 2019; Méndez, Fabra, Falcó, et al., 2021). However, around 15–20% of the fruit harvested is wasted (Munera et al., 2019), mainly due to factors associated with storage, ripening processes, fruit disease and stringent quality standard demands, thereby resulting in a huge amount of discarded fruits. Therefore, there is a need to develop holistic approaches for the management and utilization of persimmon wastes, since they can be a source of high-value compounds with different industrial applications. In particular, their high pectin and polyphenols content makes them ideal for the extraction of bioactive pectin extracts (polyphenol-rich pectin) and hydro-ethanolic polyphenol extracts (Méndez, Fabra, Falcó, et al., 2021; Méndez, Fabra, Falcó, et al., 2021), the extraction of which has been previously optimized for further applications in food-related industries. Interestingly, it was found that the polyphenol functional pectin extract extracted under severe conditions (pH 1 at 95 °C during 30 min) produced the highest pectin and polyphenol yields, with abundant non-covalent interactions, while applying less severe extraction conditions (pH 1.5 at 70 °C during 30 min) produced a pectin with intact pectin-polyphenol ester and O-glycosyl bonds (Méndez, Fabra, Falcó, et al., 2021). Pectin-polyphenol complexes are formed mainly through cooperative hydrogen bonds and hydrophobic interactions and play an important role in the regulation of the phenolic resource, for instance having better health promoting benefits than free-polyphenols since these bound polyphenols reach the colon where they are released and fermented by bacteria into absorbable metabolites (Liu, Martinez-Sanz, Lopez-Sanchez, Gilbert, & Gidley, 2017; Tomas, 2022). However, only a minor part of the existing literature on polyphenols addresses the great importance of the formation of non-extractable polyphenols (bound polyphenols) by interaction with polysaccharides (in this case pectin) (Bermúdez-Oria, Rodríguez-Juan, Rodríguez-Gutiérrez, Fernández-Prior, & Fernández-Bolaños, 2021; Méndez, Fabra, Falcó, et al., 2021; Siemińska-Kuczer, Szymańska-Chargot, & Zdunek, 2022).

In the light of the above, and based on the findings of the research group, it was hypothesized that bioactive pectins extracts may have higher antiviral activity than hydro-ethanolic polyphenol extracts with the additional advantage that they may present film-forming capacity due to the presence of pectin. Therefore, the main goals of this study were first to assess the effect of several chemical forms of polyphenols (isolated in an extract, non-covalently- bound to pectin and covalently-bound to pectin) on the antiviral properties of the extracts and, later on to investigate the film-forming capacity of the bioactive pectin extracts and to evaluate the antiviral efficacy of the developed coatings when applied to blueberries at room temperature.

2. Materials and methods

2.1. Extraction of bioactive pectins and hydro-ethanolic polyphenolic enriched extracts

Following the 3-level full factorial design carried out to optimize the extraction process of polyphenol-rich pectin extracts from persimmon fruit (*Diospyros kaki Thunb.*) (Méndez, Fabra, Falcó, et al., 2021), two different conditions were selected for this work: i) where the yield and polyphenol content were the highest (79.48 ± 0.78 mg gallic acid equivalent GAE/g pectin), with abundant non-covalent interactions (PPN) (95 °C pH 1.0 and 30 min) and ii) where the yield was high, leaving intact pectin-polyphenol ester and O-glycosyl bonds (PPC) (70 °C pH 1.5 and 30 min), (103.41 ± 0.76 mg GAE/g pectin). Similarly, hydro-ethanolic polyphenol-rich extracts (EPE) were obtained following the full central composite design carried out to optimize ethanolic heat extraction process in terms of total polyphenol content (26 ± 0.58 mg GAE/g extract) and antioxidant activity from persimmon fruit (Méndez, Fabra, Falcó, et al., 2021). Briefly, the ethanolic heat extraction was carried out at 60 °C for 1 h using a solid:liquid ratio of 83.3 g/L and a mixture of 75:25 ethanol:water solution. The extract was subsequently filtered with a muslin cloth, freeze-dried and stored in a desiccator with silica gel until subsequent characterization.

Citrus pectin (CP), obtained from Sigma-Aldrich was used for comparative purposes. The degree of esterification (DE) of CP was ~59%, as reported by Méndez et al. (2022)

2.2. Antiviral capacity-virus propagation and cell lines

Murine norovirus (MNV-1), used as a human norovirus surrogate, was propagated and assayed in RAW 264.7 cells, both kindly provided by Prof. H. W. Virgin (Washington University School of Medicine, USA). Hepatitis A virus (HAV) (strain HM-175/18f) was purchased from ATCC (VR-1402), and was propagated and assayed in confluent FRhK-4 cells (kindly provided by Prof. A. Bosch, University of Barcelona, Spain). Semi-purified MNV and HAV viruses were harvested at 2 days and 12 days after infection, respectively, by three freeze-thaw cycles of infected cells followed by centrifugation at 660×*g* for 30 min to remove cell debris. Infectious viruses were enumerated by determining the 50% tissue culture infectious dose ($TCID_{50}$) with eight wells per dilution and 20 μL of inoculum per well using the Spearman-Karber method (Pintó, Diez, & Bosch, 1994).

Extracts and pectins (EPE, PPN, PPC and CP) were dissolved in PBS at 10 mg/mL concentration. To reach a final concentration of 5 mg/mL, each solution was incubated in an equal volume of MNV and HAV suspensions (*ca.* 5–6 log $TCID_{50}$/mL, respectively) for 16 h (overnight, ON) at 25 and 37 °C in a final extract concentration of 2.5 mg/mL. Samples were then ten-fold diluted in Dulbecco's Modified Eagle's Medium (DMED) supplemented with 10% fetal calf serum (FCS) and infectivity was determined by $TCID_{50}$. Ten-fold dilutions of treated and untreated virus suspensions were inoculated into confluent cell monolayers in 96-well plates. Virus suspensions untreated in PBS under the same experimental conditions were used as a positive control. Each treatment was done in triplicate. Virus decay titer was calculated as log10 (Nx/N0), where N0 is the infectious virus titer for untreated samples and Nx is the infectious virus titer for extracts treated samples (Falcó et al., 2018).

2.3. Preparation and characterization of stand-alone films

Four different coating formulations were obtained using both persimmon pectin (PPC and PPN) by the solvent casting method: two with PPN and two with PPC, mixed with commercial citrus pectin (CP) at two different ratios, 90:10 and 80:20 (CP-PPC or PPN ratio). Control films only with CP were also prepared for comparative purposes.

To this end, 0.5 g pectin (CP, PPN or PPC) were dissolved overnight in 50 mL of distilled water using a magnetic stirrer at a controlled temperature of 30 °C until they were completely dissolved. Then, solutions were mixed at different ratios and the mixture was homogeneously spread over a Teflon plate of 15 cm in diameter and left to dry in an oven at 30 °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 with CP were also prepared for comparative purposes. It is interesting to note that neither pure PPC nor PPN films could be properly obtained (without cracks) and thus, neat polyphenol functional pectin films were not characterized. Dry films could be peeled intact from the casting surface. The obtained films were removed from the plates and equilibrated for four days in a desiccator at 23 °C and 53% relative humidity (RH) using an oversaturated solution of $Mg(NO_3)_2$. Film thickness was measured in quintuplicate using a hand-held digital micrometer (Palmer-Comecta, Spain, 0.001 mm), and the average value was used to determine the

physicochemical properties.

Sample's nomenclature was "Cx-PPx' where 'x' refers to the pectin percentage in the mixture, "C" indicates citrus pectin and PP the persimmon pectin either N or C type.

2.3.1. Morphology characterization

The microstructural analysis of the cross-sections of the dried films was carried out using Scanning Electron Microscopy (SEM) (Hitachi S-4800) at an accelerating voltage of 10 kV and a working distance of 8–12 mm. Small pieces of the pectin-based films were sputtered with a gold–palladium mixture under vacuum before their morphology was examined.

2.3.2. Optical properties of bioactive films

The transparency of the films was determined through the surface reflectance spectra in a spectrocolorimeter CM26d (Minolta Co., Tokyo, Japan), with a 12 mm diameter illuminated sample area. Measurements were taken in triplicate for each sample using white and black backgrounds.

Film transparency was evaluated through the internal transmittance (Ti) (0–1, theoretical range) by applying the Kubelka-Munk theory for multiple scattering to the reflection data. Moreover, CIE-L_{ab}* h_{ab}* C_{ab}* coordinates were obtained from the reflectance of an infinitely thick layer of material.

2.3.3. Mechanical properties

Tensile tests were carried out at ambient conditions of typically 23 (±1) °C and 54 (±2) % RH on a universal test Machine (Instron, USA), according to ASTM standard method D882.10 (ASTM D 882-02, 2002). Pre-conditioned samples were mounted in the film-extension grips of the testing machine and stretched at 50 mm/min until breaking. The tensile strength (TS), elastic modulus (E), and elongation at break (EAB) of the films were determined from the stress-strain curves, estimated from force-distance data obtained for the different films (1 cm wide and 8 cm long). At least eight replicates were obtained per formulation.

2.3.4. Water vapour

Direct permeability to water vapour (WVP) was determined from the slope of the weight gain versus time curves at 23 °C, using the (ASTM-E96/E96M, 2016) gravimetric method. For each type of sample, tests were done in triplicate and water vapour permeability was carried out at 0–75% relative humidity gradient. The films were sandwiched between the aluminium top (open O-ring) and bottom (deposit for silica gel) parts of Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium). A Viton rubber O-ring was placed between the film and bottom part of the cell to enhance sealability. Permeability cups containing silica were subsequently placed in equilibrated cabinet at 75% RH using an oversaturated solution of sodium chloride salt.

2.3.5. Water contact angle measurements

The surface hydrophobicity as the wettability of fungal-based films were measured in a DSA25 equipment (Kruss) equipped with image analysis 4021 CE software at ambient conditions. A water droplet (~3 μL) was deposited on the film surface with a precision syringe. Contact angle values were obtained by analysing the shape of the distilled water drop after it had been placed over the film for 2 s. Five replicates were analysed per film formulation.

2.3.6. Water uptake

The water absorption behaviour of the films was determined. The samples were dried prior to testing at 60 °C to constant weight. They were then placed in a 75% RH equilibrated cabinet with a supersaturated sodium chloride salt solution and gravimetric measurements were performed until constant weight. For each type of sample, the tests were carried out in triplicate. The water absorption content was calculated as a percentage of weight gained with respect to the initial weight of the sample.

2.4. Antiviral test on berries

Locally purchased blueberries were exposed to UV for 15 min in a laminar flow hood to reduce the microbial load. Then, they were inoculated by spotting 50 μL of MNV or HAV suspensions (about ca. 5–6 log $TCID_{50}$/mL) and dried under continuously circulating laminar flow hood for 45 min before the application of the coatings. Blueberries were coated by dipping in the coating solution for 2 min, let them dry for 20 min and then stored at 25 °C during 16 h and 4 days. On each sampling day, individual treated and untreated blueberries were 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. Finally, blueberries were removed from the tube and serial dilutions were performed from the resultant virus suspension. Each treatment was carried out in triplicate. Positive controls were uncoated blueberries and coated berries with commercial citrus pectin (CP), and samples coded CP90-PPN10, CP90-PPC10, CP80-PPN20 and CP80-PPC20 were tested with their respective coating forming solution. The decay of MNV and HAV titers was calculated as described above.

2.5. Statistical analysis

All statistical analysis was performed using the statistical software Statgraphics Centurion XVI® (Manugistics Inc.; Rockville, MD, USA). Statistically significant differences were determined by using one-way analyses of variance (ANOVA) and Tukey-test was used to determine statistically significant differences among means (p-value < 0.05).

3. Results

3.1. Antiviral activity of bioactive pectins

In the first part of this work, the antiviral activity of polyphenol-rich pectin extracts (PPC and PPN) were evaluated and compared with the hydro-ethanolic polyphenol extract (EPE) and commercial citrus pectin (CP). The main phenolic compound identified in the EPE extracts was the hydrolysable tannin gallic acid and others such as naringin, hesperidin or naringenin were also present, as it has been recently reported (Méndez, Fabra, Falcó, et al., 2021). Regarding the polyphenol-rich pectin extracts, the extraction conditions significantly affected the amount and type of polypohenols. PPN obtained under severe conditions displayed a very significant reduction in esterified phenolics, while non-covalently bound ("free" phenolics) significantly increased compared to those obtained under less severe conditions (PPC). Furthermore, flavan-3-ols and flavanones were only detected in PPC and not in PPN (Méndez et al., 2022).

Table 1 shows the effect of each extract against MNV and HAV at two different temperatures. Overall, polyphenol-rich pectin (PPC and PPN) exhibited the most effective antiviral activity at both temperatures, thus confirming that the presence of polyphenol-polysaccharide complexes had a positive effect on the antiviral properties, being even accentuated in those containing covalent pectin-polyphenol interactions. Higher reductions were reported for MNV and HAV after overnight (ON) incubation at 37 °C, with MNV titers decreasing under detection limits and 2.20 log $TCID_{50}$/mL for PPC and PPN, respectively. HAV titers were completely reduced under detectable limits for both PPC and PPN after ON incubation at 37 °C. Statistically significant reductions ($p < 0.05$) on MNV infectivity were observed for PPC at both temperatures, while no differences ($p > 0.05$) between PPN and PPC were reported on HAV neither at 37 °C nor at 25 °C.

Therefore, considering that polyphenol-rich pectin extracts had better antiviral properties, PPC and PPN were used to develop edible films and coatings for food preservation, with the additional advantage that the presence of pectin confers them in relation to film-forming

Table 1
Reduction of murine norovirus (MNV) and hepatitis A virus (HAV) titers (log $TCID_{50}$/mL) on bioactive pectins and hydro-ethanolic polyphenol persimmon extract at different temperatures.

Sample(*)	MNV			
	37 °C		25 °C	
	Log $TCID_{50}$/mL	**R**	**Log $TCID_{50}$/mL**	**R**
Control	6.65(0.13)[a]	–	6.62(0.47)[a]	
CP	6.28(0.14)[b]	0.37	5.57(0.13)[bc]	1.04
EPE	6.55(0.25)[ab]	0.1	6.57(0.13)[a]	0.04
PPC	<1.15 [d]	>5.50	3.16(0.07)[d]	3.46
PPN	4.45(0.12)[c]	2.2	4.87(0.56)[c]	1.75
	HAV			
	37 °C		25 °C	
	Log $TCID_{50}$/mL	**R**	**Log $TCID_{50}$/mL**	**R**
Control	4.99(0.44)[a]		5.24(0.26)[a]	–
CP	5.28(0.52)[a]	−0.29	4.70(0.33)[a]	0.54
EPE	5.32(0.13)[a]	−0.33	4.95(0.45)[a]	0.29
PPC	<1.15 [b]	>3.84	3.41(0.19)[b]	1.83
PPN	<1.15 [b]	>3.84	4.03(0.38)[ab]	1.21

Different superscripts within a column indicate significant differences among formulations ($p < 0.05$). Data reported are mean values and standard deviation (in brackets). (*) CP: citrus pectin, EPE: hydro-ethanolic polyphenolic enriched extracts; PPC: polyphenol functional pectin with intact pectin-polyphenol ester and O-glycosyl bonds, PPN: polyphenol functional pectin with abundant non-covalent interaction.

capacity.

3.2. Properties of stand-alone pectin-based films

With the aim of developing antiviral edible coatings, PPC and PPN were used as biopolymer coatings and mixed with a commercial citrus pectin at different ratios, justified by their higher antiviral properties but lower extraction yields (Méndez, Fabra, Falcó, et al., 2021) when compared to commercial pectin sources. Then, the physicochemical characterization of the developed films and the antiviral efficiency when applied as coatings onto blueberries were evaluated.

The morphology of the films was analysed by SEM and representative micrograph images of the pectin-based films after one week storage at 23 °C and 53% RH are shown in Fig. 1. As observed, commercial citrus pectin films (Fig. 1A) exhibited a smooth and homogeneous appearance with no brittle areas or bubbles, evidencing the formation of a compact arrangement of pectin chains. The incorporation of persimmon pectin extracts provided a slightly rougher structure, being more accentuated as the PPN or PPC concentration increased (Fig. 1D and E). Interestingly, blending PPN or PPC with CP gave rise to a substantially different microstructure, probably ascribed to differences in the extracted pectin structure and also to the different polyphenol-pectin interactions in both extracts. The presence of more polar compounds (phenolic acids, and specifically gallic acid) found in a higher amount in functional pectins extracted under less severe conditions (PPN) could be interacting with CP matrix through OH- bond, producing a less heterogeneous cross-section than those containing PPN. In contrast, a coarse aspect can be distinguished when PPC was mixed with commercial citrus pectin, where PPC appeared randomly distributed throughout the film cross-section (Fig. 1C and E), fact which will have a positive impact on the physicochemical properties of the film as it will be shown below.

Fig. 2 shows the spectra distribution curves of internal transmittance (T_i), used as transparency indicator, of the developed films. An increase in the Ti values is ascribed to an increase in transparency. As observed, those prepared with pure commercial pectin (CP) were the most transparent. In contrast the presence of polyphenol-rich pectin significantly reduced the transparency of the films, showing lower Ti values in all the wavelength considered. This was expected not only because of the more heterogenous internal structure, ascribed to the presence of different compounds with different refractive indices which provoked light dispersion, but also due to the light selective absorption of polyphenol compounds at low wavelengths which, moreover, are the main responsible of the brownish colour of the films.

Table 2 summarizes the optical parameters (Lightness -L*-, hue -h*ab- and Chroma –C*ab-) and the total colour differences (ΔE) obtained from the reflectance spectra of an infinite thickness film. As clearly observed, films containing polyphenol-rich pectin extracts were darker (lower L*) with a saturated brownish colour (higher C* and lower h*), which was similar to those previously reported in other biopolymer matrices (Falcó, Randazzo, Sánchez, López-Rubio, & Fabra, 2019; Moreno et al., 2020; Orqueda et al., 2022; Zhang, Jiang, Rhim, Cao, & Jiang, 2021). These differences were clearly evidenced by the increase of ΔE values in films prepared with the polyphenol-containing persimmon pectin, being accentuated as the PPN and PPC content increased.

The performance properties of the films were also characterized to assess their potential as food coatings. The mechanical properties were measured by means of tensile testing and the most representative parameters (E, TS and EAB) obtained from the stress-strain curves are gathered in Table 3. The first clear observation is that, for the films containing polyphenol-rich pectin extracts, the ones with covalently-bound polyphenols (PPC) were stiffer (higher E values) and more resistant to break (higher TS values) than their counterparts prepared with PPN. This can be correlated with the established interactions between polyphenolic compounds and pectin via ester and glyosidic linkages (Méndez, Fabra, Falcó, et al., 2021). In fact, an increase in E and TS values was observed as the PPC amount increased in the pectin-based film. Falcó et al. (2019) reported similar results for carrageenan films containing green tea extract who also attributed this behaviour (higher E and TS values and lower EAB values) to the existing polyphenol-polysaccharide interactions. Curiously, no significant differences were observed for EAB values.

With regards to water vapour permeability (WVP) values of the pectin-based films, shown in Table 3, they ranged between 1.4 and 2.1 $\times 10^{-13}$ kg-/Pa-*s*-m, which agree with those from previous studies in which pectin-based films were obtained (Aguirre-Joya et al., 2018; Bernhardt, Pérez, Fissore, De'Nobili, & Rojas, 2017; Rodsamran & Sothornvit, 2019). However, higher permeability values were previously reported for persimmon-derived pectin-based films (Matheus et al., 2021). The results evidence no major differences between samples, regardless of the presence of polyphenol compounds.

The water sorption capacity was also measured through gravimetrical tests and the results are summarized in Table 3. The presence and type of polyphenols seemed to have a notable effect as the films produced from PPC presented lower values when compared with those obtained with pure commercial pectin or mixtures with PPN. This is mostly explained by the chemical nature of the phenolic compounds and, specifically, by their polarity. The greatest reduction was seen when less polar phenolic compounds (such as flavonoids) present in the covalently bound pectin extracts (PPC sample) were incorporated in the films, in comparison with those obtained under severe extraction conditions, which rendered more polar phenolic acids (Méndez, Fabra, Falcó, et al., 2021). Furthermore, the presence of pectin-polyphenol ester and O-glycosyl bonds in the PPC sample, reduced the amount of free OH- groups thus increasing the hydrophobicity of the film.

The wettability properties of the developed pectin-based films were also determined by direct measurement of contact angles of a water drop deposited on the upper surface of samples and the results are also gathered in Table 3. Interestingly, the presence of PPC had a positive impact on the hydrophobicity of the films (increased θ values). In contrast, PPN addition provoked a significant decrease of the contact angle values, suggesting the more hydrophilic character of these films. This might be related to the differences in the chemical structure and polyphenol-pectin interactions. The presence of more polar compounds

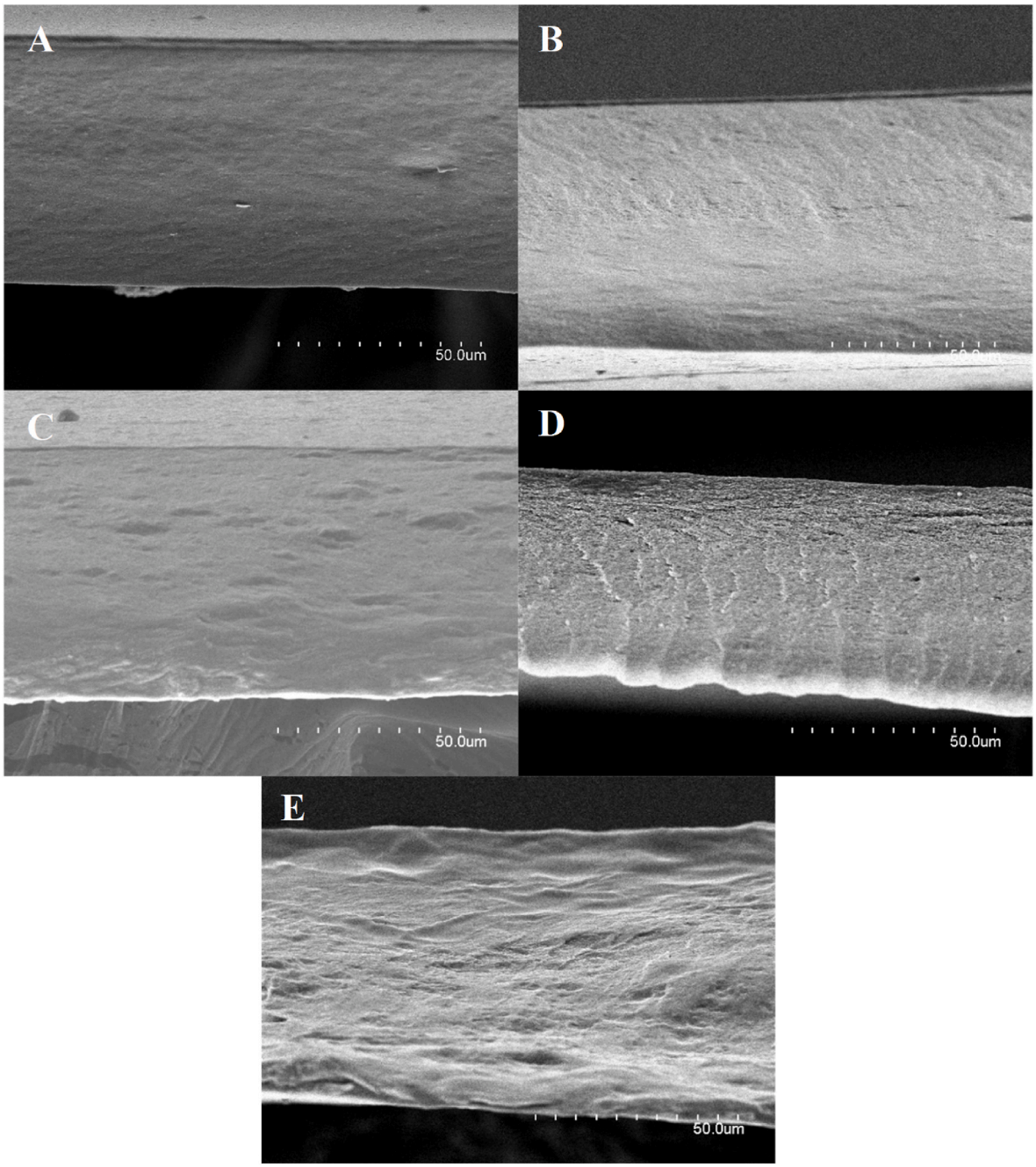

Fig. 1. Cross-section images of the developed films. (A) CP, (B) CP90-PPN10, (C) CP90-PPC10, (D) CP80-PPN20, (E) CP80-PPC20.

(phenolic acids, and specifically gallic acid) found in a higher amount in persimmon pectins extracted under less severe conditions (PPN), made the surface of the film more hydrophilic. In contrast, the less polar covalently-bound polyphenols (i.e. flavonoids aglycones) and the presence of pectin-polyphenol ester and O-glycosyl bonds found in a greater amount in PPC samples increased the hydrophobicity of the films and thus, higher contact angle values were found in the CP-PPN mixtures.

3.3. Challenge tests

Challenge tests on coated blueberries were carried out under conditions of *in vivo* storage, mimicking realistic scenarios of fresh fruit handing. To this end, each film-forming dispersion was used to treat fresh blueberries artificially inoculated with MNV and HAV and stored at room temperature (ON and during 4 days). Interestingly, as observed in Table 4, the infectivity of MNV and HAV in fresh blueberries after CP-coating treatment was reduced by 3.07 and 1.63 logs, respectively, after ON storage at 25 °C. However, the efficacy of the coatings containing pectin persimmon extract was not significantly improved, except in those containing the lower amount of PPC or PPN. The slightly higher antiviral activity in those containing 10% PPC agreed with the *in-vitro* results (see Table 1) and can be related to the presence of covalent pectin-polyphenol complexes. Surprisingly, despite of having higher PPC and PPN content, lower reductions were reported in coated blueberries. In fact, MNV and HAV titers were not significantly ($p < 0.05$) reduced for CP80-PPC20 or CP80-PPN20 coatings when compared to the commercial citrus pectin coating. It should be noted that a complete inactivation for both viruses was observed after 4 days storage at 25 °C, even in those treated with pure CP. Although the antiviral effects of bioactive polysaccharides have been mainly ascribed to the sulphated

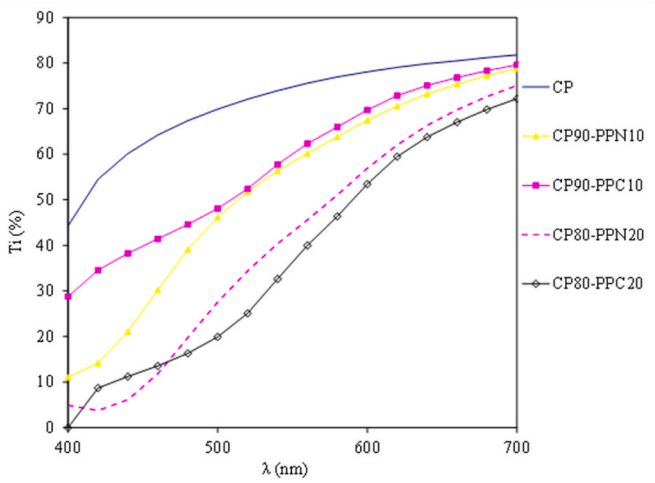

Fig. 2. Spectral distribution of internal transmittance (Ti) of the developed pectin-based films.

Table 2
Colour parameters of the developed stand-alone pectin-based films.

Films	L*	C^*_{ab}	h^*_{ab}	ΔE
CP	60.21(0.08)^{a}	19.05(0.09)^{c}	76.75(0.06)^{a}	–
CP90-PPN10	54.46(0.18)^{b}	28.31(0.18)^{a}	75(0.42)^{a}	11.01(0.13)^{a}
CP90-PPC10	47.37(0.42)^{c}	22.03(0.56)^{b}	61.19(0.36)^{c}	14.2(0.13)^{b}
CP80-PPN20	35.13(0.37)^{e}	20.02(0.38)^{c}	54(1.41)^{b}	26.37(0.24)^{c}
CP80-PPC20	37.31(0.13)^{d}	20.3(0.49)^{c}	49.46(0.53)^{d}	24.74(0.01)^{d}

Different superscripts within a column indicate significant differences among formulations ($p < 0.05$). Data reported are mean values and standard deviation (in parentheses). L* lightness, C^*_{ab} Chroma, h^*_{ab} hue. ΔE Total colour differences with respect to the control film (CP).

Table 3
Mechanical parameters (E: elastic modulus, TS: tensile strength, EAB: elongation at break), water vapour permeability (WVP), water vapour sorption (w_e) and contact angle (**θ**) of the stand-alone coatings.

Stand-alone coatings (films)*	E (MPa)	TS (MPa)	EAB (%)	w_e (g water/ 100 g sample)	θ (°)	WVP (Kg·/ Pa·s·m) x 10^{-13}
CP	4793 (437)^{b}	62.61 (11.83)^{b}	2.08 (0.34)^{a}	18.41 (1)^{a}	80.71 (0.46)^{b}	2.12 (0.33)^{a}
CP90-PPN10	4850 (219)^{ab}	43.33 (12.86)^{b}	1.25 (0.57)^{a}	17.98 (0.64)^{ab}	71.8 (0.99)^{c}	1.41 (0.26)^{a}
CP90-PPC10	5493 (274)^{ab}	67.4 (24.9)^{ab}	1.80 (0.75)^{a}	14.06 (0.31)^{d}	89.56 (1.02)^{a}	2.00 (0.08)^{a}
CP80-PPN20	4888 (305)^{b}	82.14 (19.24)^{ab}	2.48 (0.69)^{a}	16.69 (0.67)^{ab}	64.25 (1.65)^{d}	1.63 (0.05)^{a}
CP80-PPC20	6484 (1047)^{a}	94.27 (10.39)^{a}	2.40 (0.26)^{a}	15.29 (0.86)^{c}	90.07 (0.77)^{a}	1.59 (0.37)^{a}

Different superscripts within a column indicate significant differences among formulations ($p < 0.05$). Data reported are mean values and standard deviation (in parentheses). (*) CP: citrus pectin; PPC: polyphenol functional pectin with intact pectin-polyphenol ester and O-glycosyl bonds, PPN: polyphenol functional pectin with abundant non-covalent interaction.

ones (Falcó et al., 2019; Guo et al., 2022), some pectins have been reported to exert antiviral activity. For instance, Chen et al. (2019) demonstrated that pectin from *Saussurea laniceps* petals showed antiviral activity against hepatitis B virus. Similarly, de Godoi et al. (2019) reported the antiviral activity of pectin isolated from *Inga* ssp. Fruits against the herpes simplex virus type 1 (HSC-1) and the poliovirus (PV). The antiviral effect of *Houttuynia cordata* polysaccharide extract (a pectin-like acidic polysaccharide) on MNV was also evaluated by Cheng et al. (2019). Furthermore, hesperidin is the predominant flavonoid in citrus fruits with potential antiviral activity and thus, hesperidin-rich citrus pectin has also shown antiviral activity (Meneguzzo, Ciriminna, Zabini, & Pagliaro, 2020).

Table 4
Reduction murine norovirus (MNV) and hepatitis A virus (HAV) titers (log $TCID_{50}$/mL) on blueberry surfaces after treatment coatings at room temperature and different times.

Sample*	MNV			
	16 h (ON)		4 days	
	Log $TCID_{50}$/mL	**R**	**Log $TCID_{50}$/mL**	**R**
Fruit control	5.49(0.19) ^{a}	1.07	4.74(0.19)^{b}	1.82 ^{a}
CP	3.49(0.26) ^{b}	3.07	<1.15	>5.41 ^{b}
CP90-PPN10	2.74(0.73)^{bc}	3.82	<1.15	>5.41 ^{b}
CP90-PPC10	2.28(0.14)^{c}	4.28	<1.15	>5.41 ^{b}
CP80-PPN20	3.45(0.22)^{b}	3.11	<1.15	>5.41 ^{b}
CP80-PPC20	3.37(0.07)^{b}	3.19	<1.15	>5.41 ^{b}
	HAV			
	16 h (ON)		4 days	
	Log $TCID_{50}$/mL	**R**	**Log $TCID_{50}$/mL**	**R**
Fruit control	4.95(0.13)^{a}	0.04	4.99(0.19)	0.00 ^{a}
CP	3.37(0.19)^{bc}	1.63	<1.15	>3.84 ^{b}
CP90-PPN10	2.66(0.63)^{c}	2.33	<1.15	>3.84 ^{b}
CP90-PPC10	2.62(0.07)^{c}	2.38	<1.15	>3.84 ^{b}
CP80-PPN20	3.66(0.40)^{b}	1.33	<1.15	>3.84 ^{b}
CP80-PPC20	3.41(0.26)^{bc}	1.58	<1.15	>3.84 ^{b}

Different superscripts within a column indicate significant differences among formulations ($p < 0.05$). Data reported are mean values and standard deviation (in parentheses).

Coatings: CP: citrus pectin; PPC: polyphenol functional pectin with intact pectin-polyphenol ester and O-glycosyl bonds, PPN: polyphenol functional pectin with abundant non-covalent interaction.

4. Conclusions

In this work, the potential use of functional polyphenol-rich pectin extracts (PPC and PPN) and hydro-ethanolic polyphenol extracts (EPE) obtained from discarded persimmon fruits has been evaluated to develop antiviral edible coatings. Initially, the *in-vitro* assays showed that polyphenol-rich pectin exhibited the most effective antiviral activity, evidencing that the presence of polyphenol-polysaccharide complexes had a positive effect on the antiviral properties, being accentuated in those containing covalent pectin-polyphenol interactions (PPC). Therefore, PPC and PPN were used as biopolymer coatings and mixed with commercial citrus pectin at two different ratios (90–10 and 80–20, CP: persimmon pectin ratio). Addition of persimmon pectin gave rise to more coloured films with lower transparency and, the presence of covalently-linked polyphenols (PPC) produced stiffer films, with lower sorption capacity and more hydrophobic nature. The developed functional films revealed a notable antiviral activity against MNV and the HAV when applied onto blueberries. Adding 10% of PPC or PPN enhanced the antiviral activity after ON incubation at room temperature, although a complete inactivation for both viruses was observed after 4 days storage at 25 °C, even in those coated with pure CP. These functional edible coatings could be an alternative to reduce or eliminate human enteric viruses since they effectively reduce MNV and HAV titers in artificially contaminated blueberries.

CRediT authorship contribution statement

D.A. Méndez: Methodology, Investigation, Formal analysis, Writing - original draft. **I. Falcó**: Methodology, Investigation, Formal analysis **A. Martinez-Abad**: Conceptualization, Writing - review & editing. **G. Sánchez**: Methodology, Investigation, Formal analysis. **A. López-**

Rubio: Conceptualization, Methodology, Funding acquisition, Project administration, Writing- Review & Editing. **M.J. Fabra**: Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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