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Combination of ferulic acid with Aloe vera gel or alginate coatings for shelf-life prolongation of fresh-cut apples

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ABSTRACT

Weight loss, microbial spoilage and enzymatic browning are the main quality-determining processes which limit the shelf-life of fresh-cut apples. In this study, two edible coatings based on Aloe vera gel (AV) and sodium alginate cross-linked with calcium lactate (AC), with the addition of 10 mg/mL ferulic acid (FA) as a functional ingredient, were developed in order to prolong the quality and safety of fresh-cut apple discs. Texture parameters, pH and Brix values and water activity did not undergo relevant changes related to the treatments. Except for weight loss, which was significantly lower for the coated samples, the addition of FA was found to be the most relevant factor for the other investigated parameters, including the total phenolic content and the antioxidant activity measured by ferric reducing antioxidant power (FRAP). Browning was delayed by the addition of FA and also by the AV coating, while non-coated and alginate coated samples showed the highest values in early stages. Although no effect on *Saccharomyces cerevisiae* was observed, FA treatments and alginate were effective in reducing *Listeria monocytogenes* populations by 2.3 ± 0.4 log CFU / g, which contributes to an enhanced product safety.

1. Introduction

Fresh-cut apples are defined as pieces of apple that have been subjected to a physical alteration from their original form but remain in a fresh state. They are prepared for immediate consumption, packaged and should be stored under refrigerated conditions, thus providing convenience to consumers (Rojas-Graü, Garner, & Martín Belloso, 2010). The fresh-cut industry is a growing food sector as the global demand for healthy, fresh and sustainable products increases (Qadri, Yousuf, & Srivastava, 2015). However, health concerns have been associated with minimally processed fresh-cut produce because such products do not undergo any treatment that inactivates all pathogens prior to consumption. Hence pathogens introduced at any point in the production chain may be present when the produce is consumed. Fresh produce was reported to be one of the main vehicles of food-borne disease outbreaks in Europe, accounting for 8 % of all food-borne outbreaks in 2011. The most common causative agents were *Salmonella* spp., pathogenic *Escherichia coli* and norovirus (Ölmez, 2016). Moreover, growth of *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* has previously been demonstrated on fresh-cut apples (Abadias, Alegre, Usall, Torres, & Viñas, 2011). Because of their fresh nature and increased surface as a consequence of cutting processes, these products are very sensitive to food quality changes. Beside microbiological spoilage, deterioration can also occur by chemical and physical processes such as water loss, enzymatic changes (e.g. browning), oxidation and loss of cellular integrity (softening). Demands for quality retention for the benefit of longer and more global distribution chains as well as rising consumer expectations for high and lasting

Abbreviations: AC, sodium alginate cross-linked with calcium lactate; AOX, antioxidant activity; AV, Aloe vera; a_W, water activity; CFU, colony forming units; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FA, ferulic acid; FAE, ferulic acid equivalents; FRAP, ferric reducing antioxidant power; FW, fresh weight; ML, mass loss; NC, non-coated; OCLA, Oxoid Chromogenic *Listeria* Agar (OCLA); PBS, phosphate buffer solution; PCA, plate count agar; PPO, polyphenol oxidase; RH, relative humidity; TAM, total aerobic mesophylls; TPA, texture profile analysis; TPC, total phenolic content; TPTZ, 2,4,6-tris(2-pyridyl)-s-triazine; WVR, water vapour resistance; YMA, yeast and mould universal agar.

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quality have to be addressed (Wilson, Stanley, Eyles, & Ross, 2019).

The application of edible coatings is an approach to retard quality changes during storage. These coatings act as semipermeable barriers to O_2, CO_2 and water vapour, thus preventing amongst other things water loss, changes in firmness and oxidation (Raghav, Agarwal, Saini, Vidhyapeeth, & Vidhyapeeth, 2016). Furthermore, they can act as a carrier for functional ingredients. For instance, ferulic acid is an organic compound that can be derived from sustainable sources such as cereal by-products (Dapčević-Hadnadev, Hadnadev, & Pojić, 2018), has antimicrobial properties and is reported to be a powerful antioxidant (Ou & Kwok, 2004). The combination of edible coatings and ferulic acid could help to overcome the major drawbacks of fresh-cut apples, thus increasing their shelf-life and consumer safety.

Current research and development activities are focused on the improvement of the functional characteristics of such coatings according to the specific food properties (Dhall, 2013). However, the application of ferulic acid in edible coatings is still subject of research and its incorporation in edible coating has so far only been demonstrated by Alves, Gonçalves, and Rocha (2017) in a soy-protein isolate-based coating. In this study, two edible coatings were applied to fresh-cut apple discs. Firstly, *Aloe vera* gel which is a promising alternative to synthetic preservatives and has been found to have inherent antioxidant and antimicrobial properties (Asamenew, Bisrat, Mazumder, & Asres, 2011; Estepa, Micol, Alves, & Pe, 2004; Ray, Gupta, & Ghosh, 2013). Secondly, a recently improved sodium alginate coating which involves dipping the fruit in calcium lactate before dipping in alginate and then again in calcium lactate, so creating a more uniform coating on the fruit surface having specific barrier properties (Parreidt, Lindner, Rothkopf, Schmid, & Müller, 2019). Ferulic acid was separately tested as well as in combination with the edible coatings for its antioxidative and antimicrobial effect.

2. Materials and methods

2.1. Materials

For the preparation of the edible coatings, the following materials were used: sodium alginate (Halal, Manugel GHB, FMC Biopolymer Co., Philadelphia, Pa, USA); glycerol (> 99 %, FC), calcium L-lactate hydrate (> 98 %), Tween 80 (polyoxyethylensorbitan monooleate), Span 80 (sorbitan monooleate) and ferulic acid (99 %) (all supplied by Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Sunflower oil and Aloe vera used for the coating formulations were obtained from the local market. Apples (Jonagold) were purchased in a local supermarket.

For the microbial assays, Ringer solution (Sigma-Aldrich), triptone soy agar (TSA), plate count agar (PCA), yeast and mould universal media (YMA) and Oxoid Chromogenic *Listeria* Agar (OCLA) (Merck KGaA EMD Millipore, Darmstad, Germany) with chromogenic supplement (Oxoid, Thermofisher Scientific, Waltham, Massachussetts) were used.

2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, polyvinyl pirrolidone (PVPP), cistein, pyrocatechol and guayacol were acquired from Sigma-Aldrich (Steinheim, Germany). Sodium hypochlorite, peroxide hydrogen, methanol, sodium chloride, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were procured from Panreac (Llinars del Vallès, Spain).

2.2. Methods

2.2.1. Experimental design

2.2.1.1. Preparation of food samples. Apples of the variety Jonagold were purchased from a local supermarket and were stored at 5 °C until being used. Before the experiments, they were washed, disinfected with a commercial solution consisting of ethanol 23 % and propan-1-ol 35 % (w/w) and rinsed with sterile water to remove excess of the solution. Apples were cut into discs having a diameter of 2 cm and a height of 1 cm. The average weight of each apple disc (3.0 ± 0.1 g) was determined by weighing 90 discs using a Sartorius ED-153-N balance (Sartorius AG, Göttingen, Germany). Samples were prepared immediately before coating in order to prevent browning.

2.2.1.2. Coating preparation and application. In this study two edible coatings were investigated - Aloe vera gel and sodium alginate coating. A non-coated control was prepared using distilled water. Ferulic acid was used as a plant-derived antioxidant, mainly to prevent browning.

Aloe vera gel (AV) was prepared immediately after harvesting *Aloe vera* L. leaves. They were washed and disinfected with a commercial solution consisting on ethanol 23 % and propan-1-ol 35 % (w/w) and rinsed with sterile water. The outer rind was separated from the parenchyma and the mucilaginous gel was filtered to remove the fibers (Khaliq, Ramzan, & Baloch, 2019). The resultant was mixed at speed 6 with a Thermomix TM5 (Wuppertal, Germany), pasteurised at 80 °C for 5 min at speed 2 and then immediately cooled. Glycerol 1% (w/v, final solution basis) was added to a solution of 40 % AV gel prepared using sterile water.

The sodium alginate coating (AC) was prepared according to Parreidt et al. (2019). It consisted of a solution of sodium alginate in sterile distilled water (1.25 %) (w/w, final solution basis), stirred at 70 °C until complete dissolution. The flexibility of the coating was increased by using 2 % (w/w) glycerol as a plasticizer. In addition, 1 % (w/w) of Span 80 and 0.2 % (w/w) of Tween 80 were used as surfactants and 0.2 % (w/w) sunflower oil was used to increase the water barrier properties. The solution was homogenised with an Ultra-turrax ® (Miccra D-8, ART model Labortechnik GmbH & Co, KG, Staufen, Germany) at 10,500 min^{-1} for 5 min, and degassed in an Bandelin Sonorex RK 225 H ultrasonic bath (Bandelin, Berlin, Germany) at a frequency of 35 kHz for 5 min. A calcium lactate solution 2 % (w/v, final solution basis) was prepared to induce the gelling and cross-linking reaction.

Ferulic acid (FA) was added once the coatings were prepared to give a concentration of 10 mg / mL.

In total, six different variations were studied: non-coated (NC-X), non-coated with ferulic acid (NC-FA), Aloe vera gel (AV-X), Aloe vera gel with ferulic acid (AV-FA), alginate coating (AC-X) and sodium alginate coating with ferulic acid (AC-FA).

The coatings were applied as follows: For NC and AV, apple discs were dipped into the respective solutions for 10 s and drained for 90 s. For AC, the procedure described by Parreidt et al. (2019) was used in a sligthly adapted form . This involved a 10 s dipping in calcium lactate, sodium alginate and calcium lactate (in this sequence) and subsequent draining for 90 s after each dipping. After the dipping and draining cycles all the samples were dried for 35 min in a laminar flow cabin.

2.2.1.3. Storage conditions. The coated apple discs were kept in sterile petri dishes, with cams in order to prevent a gas tight packaging. The samples were stored in a cool chamber at 5 ± 0.5 °C and 50 ± 5 % relative humidity for up to 7 days. For destructive tests, a number of sample sets equal to the number of sampling days were prepared. For non-destructive analyses, tests were carried out on the same sampling sets.

2.2.2. Physical characterisation

2.2.2.1. Coating thickness. The coating thickness was determined using a stereo microscope (Leica MZ16, Leica Mikrosysteme Vertrieb GmbH, Benheim, Germany) by taking five measurements at different points along the cross-section of five samples (n = 5).

2.2.2.2. Weight loss. For weight loss measurement, samples were stored individually in sterile petri dishes. Each sampling day, five samples were

weighed and the measurements were made in triplicate (n = 15) using a laboratory balance (Sartorius Lab Instruments, GmbH & Co. KG, Goettingen, Germany). An ionizing blower (18 V, AC, 2 W, Sartorius Lab Instruments, GmbH & Co. KG, Goettingen, Germany) was used to increase the accuracy of the results by avoiding the static charge of the packaging material. The weight loss was calculated as the percentage weight loss on each sampling day (D_n) relative to the initial weight (D_0) (see Eq. 1).

$$\text{Weight loss}\left(\%\right) = \frac{\text{Weight}\ (D0) - \text{Weight}\ (Dn)}{\text{Weight}\ (D0)} \times 100 \quad (1)$$

2.2.2.3. Water activity (a_w). The water activity was determined using a dew point water activity meter model Aqualab 4TE (Metergroup, Spain). Prior to the measurement (n = 4), the meter was calibrated using suitable standards at room temperature.

2.2.2.4. Water vapour resistance (WVR). The water vapour resistance (WVR, s/cm) was calculated gravimetrically as described by Poverenov et al. (2014) with some modifications from the reference method in order to imitate storage conditions (temperature, relative humidity) as given by Eq. 2:

$$\text{WVR} = \left[\frac{\left(a_w - \frac{\%\,RH}{100}\right) \times p_{wv}}{R \times (275.15 + T)}\right] \times \left(\frac{A}{J}\right) \quad (2)$$

where, a_w is the water activity of the apple discs, % RH is the relative humidity of the climatic chamber (50 %), p_{WV} is the saturated water vapour pressure at 5 °C (8.58 mbar), R is the specific gas constant for water vapour (461.5 J /kg· K = 4.61 bar cm^3 /g K), T is the temperature of the climatic chamber (5 °C), A is the surface area of the food products (12.56 cm^2, considered as cylinders) and J is the gradient of the weight loss of the food product versus storage time (g / s). J and a_w were measured using devices that were previously described in this section.

2.2.2.5. Oxygen consumption. The oxygen consumption of the apple discs was measured in a specific oxygen measuring cell using three coated apple discs per repetition (2 replicates, 4 repetitions) (n = 8). For this purpose, the samples were weighed and stored in the closed cylindrical cell which had a volume of approximately 133 mL (measured in a specific set-up procedure by determining the pressure difference between the cell and a reference vessel and final calculation via the gas equation). The oxygen concentration inside was measured with an Oxi 340i meter (WTW, Weilheim in Oberbayern, Germany) attached to a Clark Electrode. The oxygen consumption was calculated using Eq. 3 and expressed as mg O_2 / 100 g fresh weight.

Consumed oxygen (mg O_2 / 100 g of fresh FW) =

$$\frac{0.0021 \times P \times [O2\ air - O2\ cell] \times V \times 32}{83.12 \times [273.15 + T]} \times \frac{100}{W} \quad (3)$$

where P is the atmospheric pressure when measured (mbar), O_2 is the oxygen concentration in the air or inside the hermetic cell (% saturation), V is the headspace volume (mL), T is the temperature when measured (ºC) and W is the weight of the three apple discs inside each cell (g).

2.2.3. Physicochemical properties

2.2.3.1. pH and Brix values. The pH and Brix values of the apple discs were measured at 20 °C. For each parameter and sampling day, five replicate samples were analysed three times (n = 15). The pH value was measured with a puncture probe for solid foods using an EC-30 pH meter (Phoenix Instruments, Garbsen, Germany). The Brix value was determined using a near infrared food scanner (SCiO, Consumer's Physics, Herzliya, Israel).

2.2.3.2. Measurement of colour changes. Colour changes were determined using a DigiEye imaging system (Luo et al., 2001) with an illumination cabinet and a diffuse D65 illuminator designed by VeriVide Ltd.. Images of apple discs were taken of five replicate samples (three repetitions, n = 15) on each sampling day. The colour was measured with DigiPix software which was calibrated using a table of standard colours given by the supplier. A standard circle of 1.7 cm ∅ was used to determine where the colour had to be measured. The software analysed the CIE L* a* b* coordinates, chroma and Hue angle of each measurement. The browning index (BI) was calculated using equations 4.1 and 4.2, according to Pathare, Opara, and Al-Said (2013).

$$BI = 100 \times \left(\frac{X - 0.31}{0.17}\right) \quad (4.1)$$

where

$$X = \frac{(a* + 1.75\,L*) \times a*}{(5.645L* + a* - 3.012b*)} \quad (4.2)$$

2.2.3.3. Texture profile analysis (TPA). Texture profile analysis was performed using a TA.XT Plus Connect texture analyzer (Stable Micro systems Ltd., Surrey, England) and a compression platen probe P/75. The pre-test, test and post-test speeds were 0.5, 0.5 and 1.0 mm / s, the deformation was 20 % and the trigger force was 0.05 N. For each sampling day, five replicate samples were measured (three repetitions, n = 15). The hardness, springiness, cohesiveness and chewiness were calculated according to Guiné et al. (2011).

2.2.3.4. Antioxidant activity (AOX). The antioxidant activity of the coated samples was assessed by ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazy (DPPH) scavenging activity assays, as described by Nicolau-Lapeña et al. (2019). A calibration curve was made using FA as a standard. The results are expressed as μg ferulic acid equivalents (FAE) / g FW as determined on four replicate samples with three repetitions (n = 12).

2.2.3.5. Total phenolic content (TPC). The total phenolic content of the coated apples was assessed by the Folin Ciocalteau method on the same extract used for the antioxidant activity determination. The procedure used was as described by Nicolau-Lapeña et al. (2019). A calibration curve was made using FA as a standard. The results were expressed as μg ferulic acid equivalents (FAE) / g FW as determined on four replicate samples with three repetitions (n = 12).

2.2.3.6. Polyphenol oxidase (PPO) activity. The enzymatic activity was determined by a spectrophotometric method. The enzymatic extraction was carried out by mixing 5 ± 0.5 g of the frozen product with 0.5 g PVPP and 10 mL 0.1 M phosphate buffer solution at pH 6 (PBS) with 0.05 mM cysteine in an Ultra-turrax ® Tube drive P control (IKA, Staufen, Germany) for 1.5 min at 5000 rpm. After filtration and centrifugation at 14,000 rpm for 10 min at 4 °C, the supernatant was stored in ice.

The PPO activity determination was carried out by adding 20 μL of the sample to 280 μL of 0.2 M pyrocathecol in PBS. The absorbance at 400 nm was read every 9 s for 3 min using a PowerWave HT microplate spectrophotometer (Biotek, Vermont, United States). To determine the enzymatic activity, the absorbance was plotted as a function of time and the slope of the linear regression was used to calculate the increment of optical density over time. Four replicate samples with three repetitions (n = 12) were measured and the results were expressed as Δoptical density (ΔOD) / g apple · s.

2.2.4. Microbiological investigations

2.2.4.1. Total aerobic mesophilic bacteria. TAM population counts were carried out after 1, 4 and 7 days after coating the apple discs. For this, 1 disc per repetition of three replicate samples (two repetitions, n = 6) was homogenized with 10 mL of Ringer solution with 1% Tween 80 in a Smasher AES Chemunex (Laboratorie Biomeriéux, Quebec, Canada) for 120 s at fast speed (620 strokes / min). Then, serial decimal dilutions were prepared. Plating was made in duplicate for each replication by inclusion pouring 1 mL into a Petri dish and adding 15 mL PCA. The plates were incubated for three days at 30 °C. The results are expressed as log CFU / g.

2.2.4.2. S. cerevisiae and L. monocytogenes culture preparation. Strains for microbiological assays (*Listeria monocytogenes* DSMZ 15675 and *Saccharomyces cerevisiae* DSMZ 70449) were purchased from German Collection of Microorganisms and Cell Cultures GmbH. They were cultured overnight in TSB or YMB, at 37 °C and 25 °C respectively.

2.2.4.3. Artificial inoculation of apples with S. cerevisiae and L. monocytogenes. In order to inoculate apple discs, a suspension having a concentration of 1×10^6 CFU / mL was prepared. For this, 20 mL of the overnight culture were centrifuged at 10 °C at 9000 rpm for 10 min. The supernatant was discarded. Then 20 mL sterile distilled water were added and the pellet was resuspended and centrifuged again under the same conditions. After resuspension in 20 mL of sterile distilled water, an appropriate volume was poured into 1 L of sterile distilled water to achieve the final desired concentration for the artificial inoculation. The apple discs were immersed in the microbial suspension (ratio 1:5), agitated for 2 min and then drained until a 5 % increase in weight was achieved. Finally, the samples were dried in a laminar flow cabinet at room temperature for 30 min.

2.2.4.4. Effect of the coatings on the microbial quality. The various coatings were applied as described in Section 2.2.1 on previously inoculated (with *S. cerevisiae* or *L. monocytogenes*) apple discs. Then they were stored in groups of three replicate samples for further investigation. Three samples were used for determining the initial microorganism concentration just after coating. Microbial sampling was performed twice with three replicates on each sampling day (n = 6). For this purpose, one apple disc was homogenized with 47 mL of sterile Ringer solution with 1 % Tween 80 according to the procedure previously described for TAM. Plating was done with the corresponding media (OCLA supplemented with chromogenic *Listeria* selective supplement for *L. monocytogenes* and YMA for *S. cerevisiae*). Plates were incubated for 48 h at 37 °C for *L. monocytogenes* and for 72 h at 25 °C for *S. cerevisiae.* The results were expressed as CFU / g.

2.3. Statistical analysis

The experiments followed a complete factorial design, with two factors: coating (NC, AV, AC) and antioxidant (X, FA). All data were checked for significance of the factors by applying analysis of variance (ANOVA), where interactions were also studied. The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. All statistical analyses were carried using JMP 13 software (SAS Institute Inc., Cary, USA).

3. Results

3.1. Physical characterisation of the coated samples

The pH of the coating formulations was measured before they were applied on the apple discs: AV formulations (AV-X, AV-FA) had similar pH values, averaging of 3.64 ± 0.70, while the coating with alginate (AC-X, AC-FA) resulted in higher pH values of 5.77 ± 0.02 and 4.68 ± 0.06 respectively. The coating thickness was only measured for the AC samples, as the application of AV gel with or without FA, and also the aqueous solution of FA alone, did not result in a visually observable film around the apple discs, but remained embedded in the surface. The thickness of the AC film was approx. 228.2 ± 15.5 μm, and no significant differences were observed in thickness values for AC-X and AC-FA.

Immediately after the treatments, the apple discs increased in weight due to the immersion in the solutions. After immersion in NC and AV, the apple discs gained 4.9 ± 0.6 % of their initial weight, while the AC-X and AC-FA coatings increased the weight by 16.2 ± 1.4 % and 15.3 ± 2.5 % respectively. After the coating application, the weight variation during storage of the apple discs (expressed as weight loss (%)) is shown in Fig. 1A. The time-dependent course of the weight loss of the apple discs was similar for all the treatments. The presence of FA was not a significant factor for weight loss in samples coated with the different formulations. Non-coated samples showed the highest water loss after 7 days, being 11.3 ± 0.4 % and 10.2 ± 0.6 % for X and FA respectively. Samples coated with Aloe vera without ferulic acid (AV-X) did not show statistically significant differences compared to NC treatments. The samples with AV-FA, AC-X and AC-FA coatings showed significantly lower weight loss than NC-X, being 8.8 ± 1.3 %, 8.8 ± 1.2 % and 8.2 ± 0.3 % at the end of the storage period respectively.

The time-dependent change in the water activity of the samples is shown in Table 2. Most of the coated sample discs maintained their a_w values from the beginning, except for the discs with AV coatings which started at lower a_w values of 0.987 ± 0.002 and ended at 0.992 ± 0.001. The a_w values of the other samples ranged from 0.989 ± 0.001 to 0.994 ± 0.004, with no remarkable effect of the coating, FA addition or storage day.

For calculation of the water vapour resistance values, the gradient of the weight variation over the seven days of storage and the mean of the a_w values of each treatment were used. Apart for the AV-coated samples, no differences were observed during the storage period. Fig. 1B shows the higher WVR of all coated samples with or without ferulic acid, compared to the non-coated (NC-X) samples. The NC samples had the lowest resistance to water vapour transmission. The increase in WVR averaged 12.0 ± 0.5 % for AV-X, AC-X and AC-FA and 22.8 ± 0.6 % for AV-FA. These values were significantly higher than those of non-coated samples. The implied reason for this is the coating solution. In the case of non-coated samples and the Aloe vera coated samples, the presence of ferulic acid significantly contributed to the increase in WVR.

The apple samples' oxygen consumption (mg O_2 / 100 g FW) was measured for all coating variations (Table 2). According to the statistical analysis, FA was a significant factor affecting respiration. All samples containing FA consumed five times less oxygen, and the oxygen concentration in the measuring cell after the storage period was very similar to that at the beginning. In contrast, the oxygen concentration in the measuring cells containing apple discs coated with NC-X, AV-X and AC-X had respectively decreased by 83.0 ± 1.8, 105.1 ± 17.9 and 84.1 ± 19.3 mg O_2 / 100 g FW by the end of storage period.

3.2. Physicochemical properties

The pH and Brix values of the coated apple samples were stable during storage. The average pH and Brix values of the apple discs (coated and uncoated) at the beginning of the storage period were 3.6 ± 0.2 and 11.4 ± 0.5° B respectively. The relevant values were 3.7 ± 0.2 and 10.8 ± 0.4° B after seven days of storage (data not shown).

The colour of the samples was evaluated using the browning index (BI). It was noticeable after the coating and drying process that the BI of the uncoated samples (NC-X) was 10.8 ± 1.1 (Fig. 2). That value was maintained during the storage period. The initial BI values were 5.4 ± 0.8, 4.0 ± 2.7, 4.0 ± 1.5, 5.6 ± 2.1 and 3.7 ± 1.2 for the NC-FA, AV-X, AV-FA, AC-X and AC-FA samples respectively. These values were

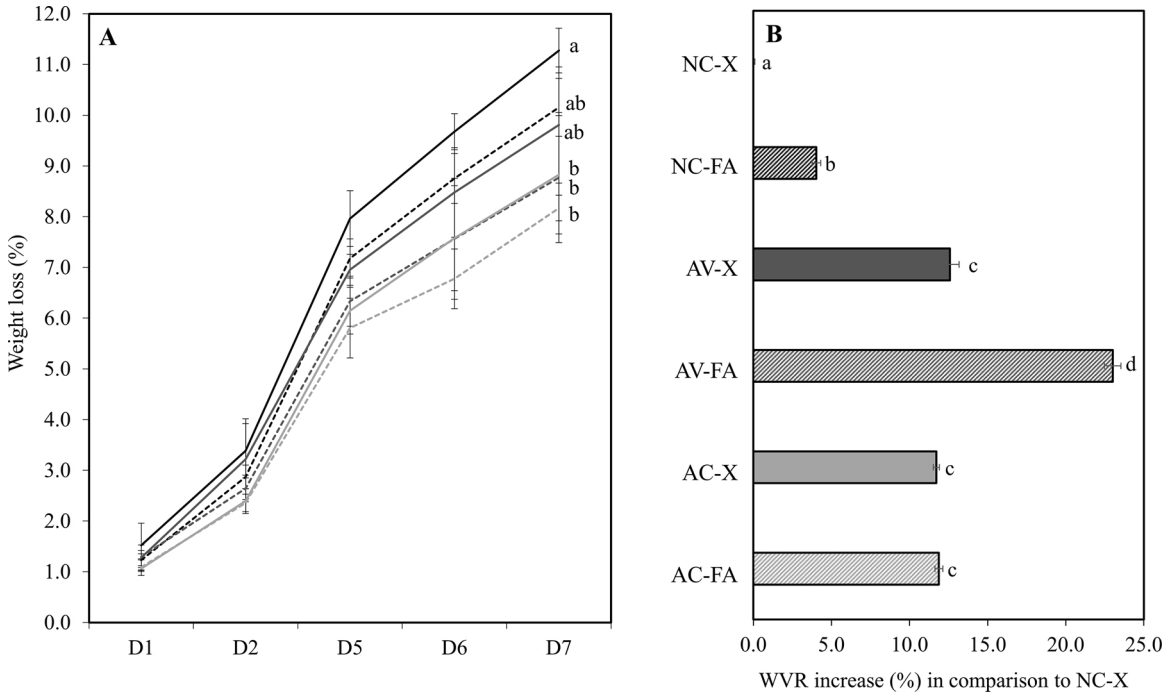

Fig. 1. (**A**) Weight loss (%) of treated apple discs during 7 days of storage at 5 ± 0.5 °C for non-coated samples (NC, ■), samples coated with Aloe vera gel (AV, ■) and samples coated with alginate and calcium lactate (AC, ■), both without ferulic acid (continuous line) and with ferulic acid (discontinuous line) (n = 15). Different letters indicate statistically significant differences between treatments at the end of the storage period ($p < 0.05$). (**B**) Water vapour resistance of treated apple discs for non-coated samples (NC, ■), samples coated with Aloe vera gel (AV, ■) and samples coated with alginate and calcium lactate (AC, ■), both without ferulic acid (solid coloring) and with ferulic acid (striped pattern) (n = 4). Values are presented as means with standard deviations. Different letters indicate statistically significant differences between treatments ($p < 0.05$).

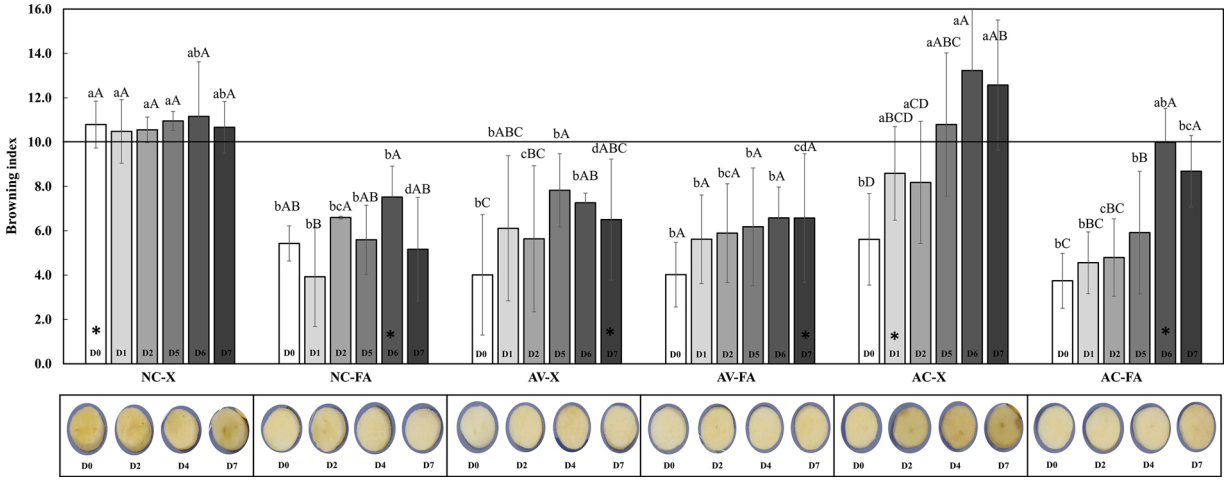

Fig. 2. Browning index of treated apple discs for non-coated (NC) samples, samples coated with Aloe vera gel (AV) and samples coated with alginate and calcium lactate (AC), both without ferulic acid (X) and with ferulic acid (FA), after different days of storage at 5 ± 0.5 °C (n = 15). The images show the evolution of the apple surface browning. Values are presented as means with standard deviations. Asterisks denote the day when significant browning was noticeable. Different lowercase letters indicate statistically significant differences between treatments for the same storage day ($p < 0.05$). Different capital letters indicate statistically significant differences between days within for same treatment ($p < 0.05$).

significantly lower than they were for NC-X samples. AC-FA samples reached a BI value of 10 at day 5 and the BI continued to increase after that. After six days of storage, all the other samples coated with FA and also AV-X showed significantly increased BI values, but none of them achieved values of 10. At the end of the storage period, the samples with the slowest browning reactions were found to be NC-FA, AV-X and AV-FA, with final BI values of 5.2 ± 2.3, 6.5 ± 2.7 and 6.6 ± 2.9 respectively.

Texture profile analyses (TPA) did not reveal an effect on the apple discs that could be attributed to the coating or to the addition of FA. On average, the initial firmness, cohesiveness, resilience and chewiness values were 161.4 ± 21.7 N, 0.6 ± 0.1, 0.4 ± 0.1, 65.3 ± 18.3 % and 51.6 ± 12.7 N respectively (see Table 1 for detailed data). No relevant differences were observed between the treatments, and after 7 days the firmness, cohesiveness and resilience values had decreased by 11.3, 15.3 and 20.7 % respectively and the chewiness had increased by 6.2 %.

The impact of the AV and AC coatings on the antioxidant properties of the samples, with and without addition of FA, is shown in Table 3.

Table 1
Initial values of pH, °Brix and texture profile analysis (TPA) of the apple discs. Results are presented as mean values with standard deviations (n = 15).

	pH		° Brix		Peak force (N)	
	D0	D7	D0	D7	D0	D7
NC-X	3.9 ± 0.5 [a]	3.8 ± 0.4 [a]	11.3 ± 0.1 [a]	10.2 ± 1.5 [a]	167.9 ± 16.1 [a]	129.8 ± 12.4 [a]
NC-FA	3.5 ± 0.2 [a]	3.5 ± 0.1 [a]	10.8 ± 0.6 [a]	10.4 ± 0.6 [a]	164.9 ± 20.9 [a]	161.0 ± 13.0 [a]
AV-X	3.4 ± 0.1 [a]	3.6 ± 0.2 [a]	11.4 ± 0.1 [a]	10.9 ± 1.0 [a]	148.9 ± 12.1 [a]	158.6 ± 7.6 [a]
AV-FA	3.5 ± 0.2 [a]	3.5 ± 0.1 [a]	12.1 ± 0.7 [a]	11.1 ± 1.1 [a]	155.1 ± 3.9 [a]	147.1 ± 2.7 [a]
AC-X	3.7 ± 0.6 [a]	3.9 ± 0.3 [a]	11.7 ± 0.2 [a]	11.2 ± 1.6 [a]	157.1 ± 5.6 [a]	145.8 ± 3.8 [a]
AC-FA	3.7 ± 0.1 [a]	3.7 ± 0.1 [a]	11.0 ± 0.6 [a]	10.7 ± 1.1 [a]	177.8 ± 15.3 [a]	117.1 ± 13.1 [a]
	Cohesiveness ([])		Resilience ([])		Chewiness (N)	
	D0	D7	D0	D7	D0	D7
NC-X	0.6 ± 0.1 [a]	0.5 ± 0.1 [a]	0.5 ± 0.1 [a]	0.3 ± 0.1 [a]	65.3 ± 0.6 [a]	58.7 ± 10.9 [a]
NC-FA	0.5 ± 0.1 [a]	0.4 ± 0.1 [a]	0.3 ± 0.1 [a]	0.3 ± 0.1 [a]	49.1 ± 0.1 [a]	54.7 ± 22.0 [a]
AV-X	0.6 ± 0.1 [a]	0.5 ± 0.1 [a]	0.4 ± 0.1 [a]	0.2 ± 0.1 [a]	49.1 ± 3.4 [a]	53.4 ± 18.4 [a]
AV-FA	0.6 ± 0.1 [a]	0.5 ± 0.1 [a]	0.4 ± 0.1 [a]	0.3 ± 0.1 [a]	40.8 ± 9.9 [a]	60.5 ± 13.3 [a]
AC-X	0.6 ± 0.1 [a]	0.5 ± 0.1 [a]	0.3 ± 0.1 [a]	0.3 ± 0.1 [a]	60.5 ± 4.7 [a]	54.1 ± 8.7 [a]
AC-FA	0.5 ± 0.1 [a]	0.5 ± 0.1 [a]	0.3 ± 0.1 [a]	0.4 ± 0.1 [a]	38.1 ± 2.3 [a]	47.5 ± 6.7 [a]

AOX studied by DPPH· assay indicates that at the beginning of the study the AOX values of samples with different treatments were not statistically different. The exception was the AC-FA coated apple discs for which the AOX values were significantly higher, reaching 1751.9 ± 134.4 μg FAE / g FW. Although some deviations were found during storage (at D4 AOX values decreased for NC-X, NC-FA and AV-X coated samples), after seven days at 5 °C the final DPPH· values were not significantly different to the initial values. Regarding the AOX capacity studied by FRAP assay, treatments with FA showed significantly higher AOX values than their counterparts without FA. Apart for a decrease in FRAP values for AC-X samples, all the other FRAP values were maintained during the storage period.

The total phenolic content (TPC) values are shown in Table 3. The highest values were observed for the NC-FA and AC-FA samples, being 23.7 ± 4.7 and 28.4 ± 0.8 μg FAE / g FW respectively, followed by 15.2 ± 2.2 and 17.9 ± 2.9 μg FAE / g FW for AV-X and AV-FA samples respectively. At the end of storage period, the differences in values between treatments were similar to those found at the beginning.

The polyphenol oxidase activity of the apple discs was determined after different days of storage (Table 3). The statistical analysis revealed that FA was a significant factor affecting the PPO activity on the first day of storage, with all samples with FA showing lower PPO activity. Even so, there was an evolution in PPO performance over time. At the end of the storage period, two different groups could be distinguished: those with lower activity, namely NC-X, and AV-X, averaging 458.0 ± 26.0 Δ OD / kg·s, and the others with higher activity of 631.6 ± 40.9 Δ OD / kg·s.

3.3. Microbiological investigations

The total aerobic mesophilic count on the apple discs was measured at days 1, 4 and 7. After 1 day of storage, TAM populations ranged between 1.3 ± 0.7 and 3.4 ± 0.2 log CFU / g (Fig. 3A). The presence of FA caused a decrease in TAM populations, and at the end of the storage period all FA samples had a two-fold lower count than samples without FA had.

The initial *S. cerevisiae* population in the apple discs after inoculation was 5.0 × 10^4 CFU / g. Immediately after the coating procedure, no changes in the population were found. Fig. 3B shows, for each treatment, the variation in the *S. cerevisiae* population from day 0 to days 4 and 7 respectively. After 4 and 7 days, no significant growth or reduction in *S. cerevisiae* count was observed. Statistical analysis revealed no significant effect of the different coating variations on the population of *S. cerevisiae* in the apple discs. *S. cerevisiae* could only grow in apple discs coated with AC-X, reaching populations of 0.9 ± 0.4 log CFU / g.

Artificial inoculation of apple discs with cells of *L. monocytogenes* resulted in a population of 7 × 10^4 CFU / g. Immediately after the coating procedure, no changes to the population were found (Fig. 3C). After a storage period of four days, the microbial count of *L. monocytogenes* decreased by 0.6 ± 0.2 log CFU / g for all treatments, including those without a coating or FA. After seven days of storage, no further inhibition was observed in the case of NC-X. The AV coating did not contribute to a decrease in the pathogen load, although some antimicrobial effect was expected for this treatment. Surprisingly, *L. monocytogenes* in AC-X samples showed a decrease comparable to the samples with FA. The samples coated with FA showed a microbial load reduction of 2.3 ± 0.4 log CFU / g compared to the first day populations.

Table 2
Values of water activity (a_w) at days 1, 4 and 7 (D1, D4, D7) as well as oxygen consumption expressed as mg O_2 / 100 g fresh weight (FW) at days 1, 2, 5 and 7 (D1, D2, D5, D7). Results are presented as mean values with standard deviations (n = 4 (a_w) and n = 8 (O_2)). Different lowercase letters mean statistically significant differences between treatments within the same day and different capital letters mean statistically significant differences between the examination days for one treatment ($p < 0.05$).

	a_w			Oxygen consumption (mg O_2/ 100 g FW)			
	D1	D4	D7	D1	D2	D5	D7
NC-X	0.991 ± 0.002 [abA]	0.993 ± 0.003 [abA]	0.992 ± 0.002 [aA]	20.9 ± 0.9 [a]	31.6 ± 2.2 [b]	59.4 ± 5.7 [b]	83.0 ± 1.8 [b]
NC-FA	0.991 ± 0.005 [abA]	0.989 ± 0.001 [bA]	0.992 ± 0.001 [aA]	10.9 ± 2.0 [b]	13.3 ± 0.4 [d]	17.7 ± 0.8 [c]	20.2 ± 0.8 [c]
AV-X	0.986 ± 0.002 [bA]	0.989 ± 0.001 [bB]	0.992 ± 0.001 [aC]	22.7 ± 2.4 [a]	56.1 ± 18.2 [a]	79.4 ± 8.3 [a]	105.1 ± 17.9 [a]
AV-FA	0.987 ± 0.002 [bA]	0.991 ± 0.002 [abB]	0.992 ± 0.001 [aB]	13.4 ± 0.1 [b]	15.4 ± 2.1 [cd]	21.1 ± 2.1 [c]	28.2 ± 7.5 [c]
AC-X	0.991 ± 0.001 [abA]	0.993 ± 0.001 [aA]	0.993 ± 0.002 [aA]	20.3 ± 1.1 [a]	29.9 ± 3.0 [bc]	62.5 ± 9.5 [b]	84.1 ± 19.3 [b]
AC-FA	0.994 ± 0.004 [aA]	0.993 ± 0.002 [aA]	0.992 ± 0.001 [aA]	12.6 ± 1.2 [b]	14.2 ± 0.2 [cd]	16.3 ± 2.5 [c]	17.3 ± 2.9 [c]

Table 3
DPPH values, FRAP values, total phenolic content (TPC, expressed as μg ferulic acid equivalents (FAE) /g fresh weight (FW)) and Polyphenol oxidase activity (PPO, expressed as increase of optical density / kg s (Δ OD / kg s)) at days 1, 4 and 7 (D1, D4, and D7). Results are presented as mean values with standard deviations (n = 12 (DPPH, FRAP, TPC) and n = 4 (PPO)).Different lowercase letters mean statistically significant differences between treatments within the same day, and different capital letters mean statistically significant differences between days for one treatment ($p < 0.05$).

	DPPH· (μg FAE / g FW)			FRAP (μg FAE / g FW)		
	D1	D4	D7	D1	D4	D7
NC-X	559.0 ± 84.5 ^{aAB}	544.7 ± 46.2 ^{abB}	754.4 ± 49.1 ^{bA}	53.6 ± 7.6 ^{abA}	56.6 ± 11.7 ^{aA}	56.6 ± 6.4 ^{bA}
NC-FA	1359.8 ± 169.9 ^{aA}	861.0 ± 167.8 ^{abB}	1210.6 ± 201.6 ^{dA}	110.2 ± 15.8 ^{dA}	82.0 ± 8.9 ^{bB}	116.6 ± 10.5 ^{eA}
AV-X	929.7 ± 204.7 ^{aA}	733.4 ± 593 ^{abB}	933.6 ± 117.7 ^{cA}	65.0 ± 10.6 ^{bA}	53.5 ± 1.2 ^{aB}	66.7 ± 3.1 ^{cA}
AV-FA	980.9 ± 192.5 ^{aA}	1078.9 ± 274.9 ^{bA}	798.0 ± 80.1 ^{bcA}	86.8 ± 16.1 ^{cA}	91.7 ± 12.9 ^{bA}	91.0 ± 3.0 ^{dA}
AC-X	677.3 ± 147.3 ^{aA}	642.9 ± 168.8 ^{aA}	423.9 ± 79.3 ^{aA}	49.1 ± 8.1 ^{aA}	46.3 ± 7.9 ^{aAB}	40.2 ± 3.1 ^{aB}
AC-FA	1751.9 ± 134.4 ^{bA}	1209.4 ± 313.1 ^{cA}	1273.8 ± 144.1 ^{dA}	135.2 ± 22.1 ^{eA}	122.6 ± 23.9 ^{cA}	112.9 ± 16.7 ^{eA}
	TPC (μg FAE / g FW)			PPO (ΔOD / kg·s)		
	D1	D4	D7	D1	D4	D7
NC-X	9.8 ± 0.7 ^{bA}	10.9 ± 2.4 ^{aAB}	11.3 ± 1.4 ^{bB}	444.5 ± 62.0 ^{abAB}	381.6 ± 120.2 ^{cA}	476.4 ± 44.9 ^{bB}
NC-FA	23.7 ± 4.7 ^{dA}	15.5 ± 2.1 ^{bB}	24.6 ± 2.0 ^{eA}	289.8 ± 45.9 ^{bA}	551.2 ± 45.9 ^{abB}	592.3 ± 5.8 ^{abB}
AV-X	15.2 ± 2.2 ^{cA}	12.0 ± 1.2 ^{aB}	13.0 ± 0.3 ^{cAB}	367.6 ± 13.6 ^{bA}	535.9 ± 18.4 ^{abcB}	439.6 ± 64.9 ^{cB}
AV-FA	17.9 ± 2.9 ^{cA}	15.8 ± 2.9 ^{bA}	17.8 ± 1.2 ^{dA}	405.1 ± 89.5 ^{bA}	448.2 ± 109.3 ^{bcA}	687.8 ± 109.3 ^{aB}
AC-X	6.4 ± 0.9 ^{aA}	10.2 ± 0.2 ^{aC}	7.7 ± 0.2 ^{aB}	597.2 ± 22.3 ^{aA}	617.7 ± 22.3 ^{aA}	632.2 ± 95.9 ^{aA}
AC-FA	28.4 ± 0.8 ^{eA}	27.6 ± 3.0 ^{cA}	24.0 ± 1.0 ^{eB}	447.1 ± 67.8 ^{abA}	441.9 ± 72.5 ^{bcA}	614.2 ± 23.8 ^{aB}

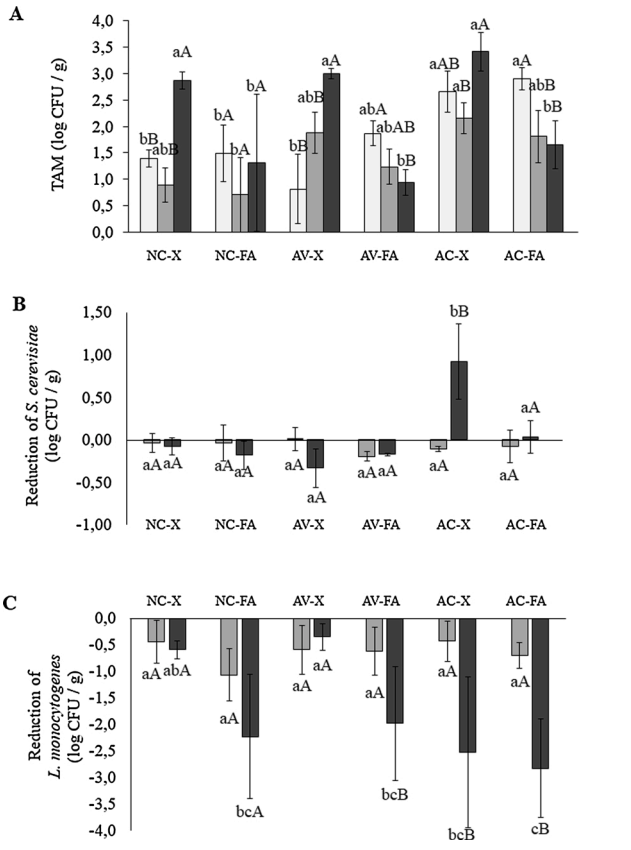

Fig. 3. (A) Total aerobic mesophyll count for non-coated (NC) samples, samples coated with Aloe vera gel (AV) and samples coated with alginate and calcium lactate (AC), both without ferulic acid (X) and with ferulic acid (FA), after storage at 5 ± 0.5 °C on day 1 (■), day 4 (■) and day 7 (■) (n = 3). (B) *Saccharomyces cerevisiae* and (C) *Listeria monocytogenes* reduction, compared to the respective initial populations immediately after application of the coatings, after storage at 5 °C on day 4 (■) and day 7 (■) (n = 6). Values are presented as means with standard deviations. Different lowercase letters indicate statistically significant differences between treatments for the same storage day ($p < 0.05$). Different capital letters indicate statistically significant differences between days for the same treatment ($p < 0.05$).

4. Discussion

In this study, two edible coatings based on sodium alginate and Aloe vera, with and without the addition of ferulic acid, were investigated. The coating thickness in our study was higher than that reported by Rojas-Graü, Tapia, Rodríguez, Carmona, and Martin-Belloso (2007) and was 132.5 ± 20.5 μm for alginate coated fresh-cut apples. This may well be attributed to the double dipping in calcium lactate which implies two cross-linking steps. This could impact sensory aspects, for example the mouth-feel. However, the thickness of the coating is within the range published in the literature for edible coatings, namely < 300 μm (Pavlath & Orts, 2009).

The weight loss, colour change and microbial growth were the three main quality parameters chosen for investigation in this work. Weight loss is expected to occur during the shelf-life of fresh-cut fruit. The main cause is water loss, caused by the migration of water from the fruit to the ambient air (Soliva-Fortuny & Martín-Belloso, 2003). In the case of the uncoated samples (NC-X) which served as a control, the weight loss was 11.3 ± 0.4 % after seven days of storage. Edible coatings can retard this process, by creating a semi-permeable barrier to water vapour. In this study, the treatments that offered the best solution for reducing the weight loss of apple discs (which at the end of storage averaged 8.6 ± 0.4 %) were AV-FA, AC-X and AC-FA. Aloe vera gel alone has also demonstrated its ability to reduce weight loss in white button mushrooms coated with 50 % AV gel (Mirshekari, Madani, & Golding, 2019) and peaches coated with 20 % AV gel (Hazrati et al., 2017). AC has also been reported to decrease weight loss in fresh-cut apples (Olivas, Mattinson, & Barbosa-Cánovas, 2007) and in other fruits such as plums (Valero et al., 2013). The measured a_w values agree with those reported in the literature (Schmidt & Fontana, 2008). However, the observation that the water activity did not change significantly while some of the samples (such as the non-coated ones) had lost about 11 % of weight could be explained as follows: The a_w value represents the free or non-chemically bound water in the food product. As the moisture content and the water activity are not linearly correlated, it is possible that even though a product losses a certain percentage of water, the water activity is still not affected. For instance, the study carried out by Chambi, Lima, and Schmidt (2016) have shown that the gradients of moisture and water activity of melon do not have the same slope and that different moisture contents in the sample could show similar water activities. Polysaccharides are reported to have lower water vapour barrier properties than other materials used for edible coatings such as lipids and some proteins (Vargas, Pastor, Chiralt, McClements, & González-Martínez,

2008). In this study, we observed significant differences in the water vapour resistance between the coated and uncoated samples. The coatings AV-X, AC-X and AC-VA increased the WVR by approximately 12.1 ± 0.5 % and the value for AV-FA was 22.8 ± 0.6 % higher than it was for NC-X samples. This may explain the reduction in weight loss of coated samples in this study. Although the presence of FA decreased the weight loss, albeit not significantly, of the samples compared to their non-FA equivalents, the presence of FA in the coatings increased the WVR of NC and AV coated samples compared to their counterparts without FA. This could be explained due to the fact that the WVR calculation takes into account not only the weight loss gradient (which was already different but not significantly) but also the a_w value. Meanwhile, in the case of the alginate coatings (AC), WVR was not altered by the presence of FA. The effect of FA was negligible for both the weight loss and the water activity, not having an impact on this parameter. This effect of FA has not been described in the literature until now as FA has been reported to not easily disperse along the surface of fruit and it is recommended to be incorporated within an edible matrix (Alves et al., 2017). When FA is added to a polymer coating, WVR values can increase because it facilitates the cross-linking of the polysaccharides (Cao, Fu, & He, 2007).

Regarding quality parameters, the pH values of the apples used in this study agreed with those in the literature for this variety (3.2–3.9) but the Brix values were lower than the reported values (12–18 ºB) (Lammertyn, Nicolaï, Ooms, De Smedt, & De Baerdemaeker, 1998). This could be related to the maturity of the fruit, which affects the soluble solids content and other quality parameters such as the titratable acidity (Herregods & Goffings, 1993). The study of the texture using TPA analysis was carried out because the parameters measured with this method have shown good correlation with sensory evaluations (Li et al., 2017). The observed variations in the investigated parameters are attributed to the action of pectic enzymes. After cutting operations, the mixing of substrates and enzymes (which normally are separated) initiates reactions which would normally not occur at such high rates (Toivonen & Brummell, 2008). Even though edible coatings could help in preserving the turgor pressure of the vegetable tissue, a texture enhancer is often added to the coating. The most common enhancers are calcium salts. Calcium ions interact with pectic polymers to form a cross-linked network that increases the mechanical strength (Rojas--Graü, Soliva-Fortuny, & Martín-Belloso, 2009). In fact, FA has been reported to maintain the firmness of blueberries which were dipped in an aqueous solution of 0.5 g FA / L and stored for 15 days. However, the underlying mechanism was not described (Xu & Liu, 2017). In another study, dipping apple slices into a formulation of ferulic acid at 4 g / L was found to be the most effective way to maintain their texture (Alves et al., 2017).

Colour maintenance was considered one of the main focuses of this study as it can affect consumers' buying intention, as it is an indicator of freshness and flavour quality (Barrett, Beaulieu, & Shewfelt, 2010). The browning index (BI) is used as an indicator of colour changes in fresh-cut apples (Lunadei, Galleguillos, Diezma, & Lleó, 2010). In the case of the uncoated samples, the BI increased during the first 35 min and then remained constant throughout the storage period. As no coating and no antioxidant were applied, we considered the mean value as the standard browning that can occur in this variety of apples. The application of the AC-X coating was not able to delay browning for more than four days. Bertrand, De Jesus Raposo, De Morais, and De Morais (2015) showed that the effect of an alginate coating 2 % (w:v) was negligible when compared with that of anti-browning agents incorporated into the coating on fresh-cut apple slices. The AV coating led to retarded browning and apple samples did not reach the browning levels of the control even after the 7th day of storage. Indeed, AV gel is reported to contain a number of antioxidant compounds, including aloe-emodin, anthraquinones and acemannan, which could contribute to colour preservation. Other reports have already described this effect on fresh and fresh-cut fruit and vegetables, including guava, plums and mango (Martínez-Romero et al., 2017; Nasution, Ye, & Hamzah, 2015; Pérez, Aristizábal, & Restrepo, 2016). The samples to which FA was added showed good retention of colour, especially NC-FA and AV-FA, which retained their initial BI values for the 7 days. Ferulic acid has been reported to be a powerful antioxidant (Ggaf, 1992; Kumar & Pruthi, 2014) and has been used in edible coatings for that purpose (Baraiya, Ramana Rao, & Thakkar, 2016; Fabra, Hambleton, Talens, Debeaufort, & Chiralt, 2011). Regarding the antioxidant activity of the coated samples, FA was the main factor that contributed to the increase in the AOX by DPPH· and FRAP values. The AV gel also caused an increase in the AOX values of coated apple discs, due to the high content of antioxidants. However, it must be noted here that at day 4 of the storage period, the AOX values (DPPH·and FRAP) had decreased for the samples NC-X, NC-FA and AV-X. The reason for this has yet to be clarified. Although there is a clear correlation between the AOX values and the total phenolic content in this study, their variations cannot be explained by PPO activity. Other authors have reported a decrease in antioxidant activities followed by an increase, depending on the matrix (Kevers et al., 2007) or the coating treatments (Oms-Oliu, Soliva-fortuny, & Martín-Belloso, 2008). The phenolic and other antioxidant compounds (e.g. organic acids) have intricate pathways and can be involved in enzymatic reactions. For this reason, variations in the enzyme content (e.g. lipoxygenase, catalase, peroxidase) should be further assessed in order to identify their interactions with the different coatings and with the FA. The highest total phenolic contents were found in NC-FA and AC-FA samples, followed by AV-X and AV-FA samples. This is explained by the presence of FA, which is a phenolic acid, and the phenolic compounds in AV gel. Coatings such as alginate have no remarkable effect on the total phenolic compounds in treated apples (Cofelice, Lopez, & Cuomo, 2019). The observed increase in phenolic compounds in most of the coating variations could be attributed to activation of phenylalanine ammonia lyase after cutting the apples, with consequent formation of phenols as a defence mechanism against pathogens or water loss (Reyes, Villarreal, & Cisneros-Zevallos, 2007). After cutting the plant tissue, enzymatic browning by polyphenol oxidase (PPO) is triggered by the decompartmentation of cells, which brings the enzyme and the substrates into contact. PPO has been directly related to the browning of fresh-cut apples but is dependent on the amount and type of polyphenols (Ferreira-Holderbaum, Kon, Kudo, & Guerra, 2010). Shannon and Pratt (1967) proposed that FA may act as a competitive inhibitor of apple PPO, preventing the binding between substrate and enzyme by occupying the latter's active sites. Contrary to what was expected, the increase in PPO activity after seven days of storage for samples containing FA was 104.4, 69.8, and 37.4 % for NC-FA, AV-FA and AC-FA samples respectively, while the corresponding non-FA counterparts showed increases in PPO activity of 7.2, 19.8 and 5.9 %. This increase in PPO activity is not reflected in the BI of the samples, so we hypothesise that, despite the higher PPO activity, the browning could have been reduced by donation of an electron to the intermediate quinone of other compounds present in the matrix and in the coating (Nirmal & Benjakul, 2009).

The oxygen consumption of a product can be determined by periodic measurement of the oxygen concentration within a hermetic cell (Saltveit, 2000). Edible coatings are reported to act as semi-permeable barriers for gases, decreasing the amount of O_2 and CO_2 that is exchanged between the product and the ambient environment, creating a modified atmosphere within the coating (Dhall, 2013). However, in this study we did not observe a reduction in the oxygen concentration due to the coating, but rather to the presence of FA: samples with FA did not consume as much O_2 as samples without FA. One hypothesis to explain this behaviour is that FA inhibits the PPO activity of the samples, and this enzyme does not use the available O_2. However, PPO activity increased more in samples with FA than without FA, which indicates no inhibiting effect. To try to respond this decrease in oxygen consumption, we investigated the correlation between the oxygen consumption and the concentration of total aerobic bacteria in the fruit. Although AC samples showed the higher TAM at the beginning of the storage period,

FA decreased their growth during the 7 days of storage. Samples without FA had significantly higher microbial populations needing oxygen for their metabolism. This led to the assumption that the microbial load mainly contributed to the observed oxygen consumption.

S. cerevisiae was studied because it is a food-borne yeast typically found in apple juice, arising from the processing of the fruit (Guerrero-Beltrán & Barbosa-Cánovas, 2005). Therefore, it was used as an example of an alternative microorganism that can occur in fresh-cut apples. After dipping in the coating solutions, the populations were maintained, indicating no microbiocidal effect of the coatings. In this study, the growth of *S. cerevisiae* was only observed in one set of samples (AC-X), which was attributed to the higher pH value of the coating (5.7) and the absence of FA, enabling suitable conditions for propagation of *S. cerevisiae*. In contrast, some authors have reported an inhibitory effect of FA against *S. cerevisiae* at doses above 250 ppm (Baranowski, Davidson, Nagel, & Branen, 1980).

L. monocytogenes is a species of food-borne pathogenic bacteria that can even grow at low temperatures, and represents a problem for produce stored under refrigeration (4 °C) (Qadri et al., 2015). Even though there have been no outbreaks associated with *L. monocytogenes* and whole apples (Harris et al., 2003), the potential growth of food-borne pathogens is greater on fresh-cut produce than on produce with an intact peel, because these products are minimally processed and there are more nutrients available on the cut surface (Conway et al., 2000). In this study, a decrease in the population of *L. monocytogenes* was found in all the samples. We attributed this to the pH value of the apples which was lower than 3.9, the value reported as the limit for its growth (European Union Reference Laboratory for *Listeria monocytogenes*, 2014). The Aloe vera coating was investigated in this study because of its reported antimicrobial activity, especially against epiphytic microbiota (Zapata et al., 2013). Under the experimental conditions used here, AV alone did not cause any decrease in the *L. monocytogenes* population. Various reasons could explain this, such as a low concentration of antimicrobial compounds (namely anthraquinones and aloe-emodin) in this batch of AV gel compared to those reported in literature (Pellizoni, Ruzicková, Kalhota, & Lucini, 2016) or the resistance of the *L. monocytogenes* strain used in this study to those compounds. Unlike the Aloe vera coated samples, the alginate coating seemed to have an inhibiting effect *per se*, even though alginate has not been reported to have any significant bacteriostatic effect. The addition of FA, as expected, promoted a decrease in the population of *L. monocytogenes* on apple discs after seven days. FA has previously been reported to have antimicrobial effects against *L. monocytogenes* (Pernin, Guillier, & Dubois-brissonnet, 2019). The mode of action involves two mechanisms. Firstly, the intercellular dissociation of the acid causes acidification of the cell cytoplasm and the efflux of K^+ ions leading to the eventual death of the microorganisms. Secondly, the intercalation of the acid in the phospholipid layers of the membrane of the microorganism could also cause a disturbance of the Van der Waals interactions, inhibiting the transport of substrates used by the key enzymes of the microorganism (Pernin, Bosc, Maillard, & Dubois-Brissonnet, 2019).

5. Conclusions

Enzymatic browning and weight loss are two processes that contribute to quality changes in fresh-cut produce. They are also considered as freshness indicators by consumers. In this study, the application of edible Aloe vera and alginate coatings with the addition of ferulic acid were evaluated as ways to delay quality changes and ensure the safety of fresh-cut apples.

The application of Aloe vera gel and alginate-based coatings led to a decrease in weight loss of the samples, whilst the texture, pH, Brix (°) and a_w values were not affected by any of the treatments. However, the factor that had the greater impact on increasing the shelf-life of fresh-cut apples was the incorporation of ferulic acid. This compound, which can be derived from sustainable natural sources, has delayed the browning in the samples for seven days (same effect was also achieved by Aloe vera gel alone). Furthermore, ferulic acid and the alginate coating significantly decreased artificially inoculated *L. monocytogenes* populations after seven days of storage. However, the relationship between some of the studied parameters, including the coatings with the respiration, PPO enzymatic activity or antioxidant evolution of the samples still needs to be investigated in more detail.

Nevertheless, the addition of ferulic acid to the edible-coatings has proven to be a promising approach for enhancing the quality and safety of fresh-cut produce.

CRediT authorship contribution statement

Iolanda Nicolau-Lapeña: Conceptualization, Investigation, Writing - original draft. **Ingrid Aguiló-Aguayo:** Conceptualization, Validation, Supervision, Writing - review & editing. **Bernd Kramer:** Conceptualization, Validation, Supervision. **Maribel Abadias:** Methodology. **Inmaculada Viñas:** Methodology. **Peter Muranyi:** Conceptualization, Validation, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interests.

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