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Aloe-Based Edible Coating to Maintain Quality of Fresh-Cut Italian Pears (*Pyrus communis* L.) during Cold Storage

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Abstract: Pear fruits are known for their antioxidant and nutritional characteristics. However, they are very susceptible to rapid decay. Edible coating (EC) represents a good strategy to maintain postharvest quality. The effects of two EC in slowing down the senescence processes in fresh-cut 'Coscia' pears were investigated: EC1 (*A.* vera gel, hydroxypropyl-methylcellulose and pomegranate seeds oil (PSO), EC2 (*A.* vera gel and hydroxypropyl-methylcellulose). Weight loss, firmness and colour decrease more slowly in both EC-treated than in untreated (CTR) slices; soluble solid content increases faster in CTR, indicating a faster ripening process. The specific investigation of undesired microorganisms did not generate any colony in all analysed samples. Sensory analysis confirmed that the tasters preferred the EC2-treated samples, as they were the only ones that did not show undesirable flavours until the last day of storage.

Keywords: Aloe vera gel; post-harvest; bio-based films; hydroxypropyl methylcellulose; pomegranate seed oil

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1. Introduction

European pear fruit (*Pyrus communis* L.) belongs to the *Rosaceae* family and is known worldwide for its flavour and crispness but is very susceptible to spoilage. In particular, the respiration rate and ethylene production trend are typical of climacteric fruits [1,2].

In general, pear fruit are an excellent source of antioxidant properties, which are important for the human diet [3]. In fact, ascorbate is regenerated by dehydroascorbate reductase (DHAR) using reduced glutathione [4]. Pear fruit is consumed as a fresh product or in the form of juice, marmalade or as dehydrated fruit [5]. Recently, fresh-cut pear has become an important food category which grows quickly due to its freshness, nutrition and convenience. These products have the attributes of convenience and fresh-like quality.

Therefore, loss of quality characteristics can be avoided or prevented by various methods, such as reducing the pH on the surface of fruit to avoid the development of microbial spoilage; reducing the CO_2 partial pressure or addition of certain inhibiting agents [6].

Concerning CO_2 partial pressure, it has been observed [7,8] that high CO_2 concentrations lead to disorders such as core browning. Furthermore, Peppelenbos and Oosterhaven [9] suggested that internal browning is probably due to the reduction of adenosine triphosphate (ATP) levels below the minimum values required for cell membrane maintenance activities, as shown by Rawyler et al. [10] in potato cell cultures.

On the other hand, addition of natural anti-browning compounds, such as antioxidants, chelating agents and acidifiers, is the most commonly used method to delay the senescence processes [11]. Various compounds, such as citric acid (CA) and ascorbic acid

(AA), calcium chloride, sodium carbonate, ethanol and cysteine, cinnamic acid, P-coumaric acid and ferulic acid, alginate, pectin, xanthan gum or gellan gum, have been used as functional edible coating ingredients in many fruit [12–16]. However, with regard to pear fruits, few edible coatings have been applied. In particular, zein and oleic acid [17], vegetable oil [18], shellac, Semperfresh^{TM} and carboxymethyl-cellulose [19], were tested in minimally processed pears, while Nandane et al. [20] tested an edible coating based on hydroxypropyl-methilcellulose (HPMC), potassium sorbate and olive oil on whole pear fruit stored at room temperature, but always with the difficulty of reducing browning.

A widely used component of edible coatings has been Aloe vera gel, which has shown a positive effect in fresh-cut fruits in order to reduce respiration rates, ethylene production, weight, firmness, color loss and microbial load, due to its antibacterial and antifungal activity and did not affect natural taste of fruit [21–25].

However, as has been reported by some authors, high concentrations of Aloe vera gel can alter the taste of processed fruit, limiting its purchase and consumption. This could lead to negative effects, such as the production of waste on the market [26–28]. The essential characteristics of an edible coating are that it is transparent and must be free of odour and taste, oil-resistant and water-soluble. Most importantly, it must also be safe in food (FAO/WHO Expert Committee on Food Additives and Contaminants—JECFA) and chemically stable [29,30]. Concerning the application of hydroxypropyl methylcellulose (HPMC) in edible coating, this seems to delay loss of flesh color, weight and firmness in fresh-cut fruit [31].

The important additives of edible coatings are the fungicide and antioxidant agents. For this purpose, essential oils have been extensively tested [32–34] in several fruit, but there are no studies concerning the application of pomegranate seeds oil (PSO) on fresh-cut 'Coscia' pear. Extracted oils, instead, are natural substances derived from fruit and vegetables. They are designated as Generally Regarded as Safe (GRAS) by the United State Food and Drug Administration (FDA). In the United States, according to 21CFR182.20 and 21CFR582.20, essential oils, oleoresins (without solvents) and natural extracts (including distillates) of pomegranate (*Punica granatum* L.) are considered GRAS for their use in food for human consumption, pharmaceuticals and related products. Indeed, due to its antioxidant and anti-inflammatory properties, extracts from various parts of *Punica granatum* have been studied [35,36] for use as alternative or therapeutic treatments (such as herbal medicines or dietary supplements) for burns, oral hygiene, neurodegenerative conditions, seizures, diabetes, acute pancreatitis, lung injury, myocardial infarction and various cancers [37,38]. The juice and extracts have also been studied for use as antifungal and antibacterial treatments [39], in particular against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli* and against different plant pathogens [40,41].

The PSO has also been used to improve the properties of biopolymer films used in food packaging [42] and could be a natural additive to reduce quality loss during storage while maintaining high nutritional parameters [43]. To date, there are no studies concerning the effects of PSO addition in edible coating on fruit quality attributes and storage conditions.

The aim of this work was to determine the effects of two edible coating treatments, in slowing down all the senescence processes in fresh-cut 'Coscia' pears cold-stored for seven days: (1) Aloe vera gel, hydroxypropyl-methylcellulose and pomegranate oil; (2) Aloe vera gel and hydroxypropyl-methylcellulose.

2. Material and Methods

2.1. Vegetal Material

Twenty kg of 'Coscia' pears (*Pyrus communis* L.), selected for uniformity of size and absence of defects, were harvested at the commercial maturity (firmness 4–4.5 Kg/cm^2) in Torrenova (ME) 38°05′18.56″N 14°40′18.08″E.

2.2. Extraction of Aloe Vera Gel and Edible Coating Formulation

One kg of mature Aloe vera leaves were collected in the experimental field of the University of Palermo and washed under tap water. The gelatinous parenchyma was separated from the leaves with a stainless-steel knife, removing the outer epidermis. It was crushed with an ultra-Turrax T25 (Janke and Kunkle, IKa Labortechnik, Breisgau, Germany) for 5 min at 24.500 rpm to form a homogeneous substance and was filtered through a press filter containing five micron filter papers in order to remove the fibrous part. A total of 500 mL of extract was obtained. Based on previous research [17,44], 120 mL of Aloe vera gel was diluted in 300 mL of water for sensory acceptability, due to a bitter taste that occurred at higher concentrations.

A commercial pomegranate seed oil (PSO) (www.lerboristeria.com© Cagliari—Italy accessed date 9 September 2020) obtained by cold mechanical pressing without the use of solvents and without refining was used. The degree of purity allows it to be certified for both cosmetic and food use.

Two edible coating (EC) were tested:

- EC1: 120 mL Aloe vera gel + 6 g HPMC + 3 g PSO were dissolved in 300 mL of water;
- EC2: 120 mL Aloe vera gel + 3 g HPMC were dissolved in 300 mL of water.

Antioxidant agents (citric acid and ascorbic acid) were added to all solutions at a rate of 3 g each. The solutions were kept at 40 °C for 90 min and then homogenized at 3.000 rpm for 20 min with ultra-Turrax.

The samples treated with the edible coating were compared with an uncoated sample (as control—CTR) to highlight the main differences.

2.3. Experimental Design

In the work area, all utensils and surfaces were previously washed and sterilised. The temperature inside the room was set at 4 °C to avoid bacterial proliferation and the fruit were first washed under tap water and then immersed in chlorinated water (100 μL-L^{-1}) for 5 min, according to methodology reported by Arias E. et al., [19]. The whole fruit were then air-dried for 20 min. The pears were then peeled and cut into 4 slices with a sterilised stainless-steel knife and the core was removed by means of a pear corer tool.

First of all, untreated slices (CTR) were retained. After that, the two edible coatings was applied to the remaining pear slices by spraying, using an airbrush (0.8 mm nozzle) supplied with N_2, for 2 min per lot. Each slice was covered with about 0.3 mm of coating, based on the time of spraying and the fruit weight. All coated slices were then allowed to dry for 5 min and, in general, four slices (approx. 100 g) were stored in PET (polyethylene terephthalate) trays.

Thirty-six trays per sample (CTR, EC1 and EC2) were stored at 4 ± 1 °C and 90 ± 5% relative humidity (RH).

Physico-chemical, microbiological and sensory analyses were carried out on day 0 (d0—as fresh product), day 2 (d2), day 4 (d4) and day 7 (d7) in three replicates per treatment.

2.4. Physico-Chemical Analysis

The difference in weight of each tray was measured throughout the storage period using a digital scale of decimal precision (Gibertini, Novate Milanese, Italy) and the values were expressed as a percentage of weight loss:

$$\text{Weight loss (\%)} = [(Wi - Wd)/Wi] \times 100$$

where Wi is the initial weight and Wd is the weight measured during cold storage.

Flesh firmness was determined using a TR5325 digital penetrometer (Turoni, Forlì, Italy) with an 8 mm diameter tip and expressed as (kg m^{-2}).

A Minolta colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Tokyo, Japan) was used to determine lightness (L*), red tendency (a*) and yellowness (b*). Prior to the analysis of the samples, the instrument was calibrated using a standard white plate.

Browning index (BI) was determined, following the formula of Ruangchakpet A. and Sajjaanantakul T. [45]:

$$(BI) = [100\ (x - 0.31)]/0.17]$$

where $x = (a^* + 1.75\ L^*)/(5.645\ L^* + a^* - 0.3012\ b^*)$.

Total colour difference (ΔE) was determined using the formula:

$$\Delta E^* = [(L2^* - L1^*)2 + (a2^* - a1^*)2 + (b2^* - b1^*)2]1/2$$

From the juice extracted from the pear slices, the soluble solid content (SSC) was determined using an ATAGO digital refractometer (Atago Co, Ltd., Tokyo, Japan) at 20 °C and expressed as °Brix.

2.5. Microbiological Analysis

Twenty-five grams of coated and untreated slice pear samples were first homogenized in 225 mL Ringer's solution (Sigma-Aldrich, Milan, Italy) by a stomacher (BagMixer® 400, Interscience, Saint Nom, France) for 3 min at the highest speed (blending power 4) and then serially diluted (1:10) into 10 mL-volume test tubes homogenized by vortex. Cell suspensions were analysed for the following undesired (spoilage and pathogenic) microbial groups: total mesophilic aerobic microorganisms (TMM) on (PCA) and total psychrotrophic microorganisms (TPM) on Plate Count Agar (PCA), incubated at 30 °C for 72 h and at 7 °C for 7 d, respectively; *Pseudomonas* spp. on *Pseudomonas* Agar Base (PAB) added with Fucidin Cephaloridine supplement (CFC), incubated at 25 °C for 48 h; members of the *Enterobacteriaceae* family on Violet Red Bile Glucose Agar (VRBGA), incubated at 37 °C for 24 h; yeasts and moulds on Yeast extract Peptone Dextrose (YPD) agar supplemented with chloramphenicol (0.1 g/L) to prevent bacterial growth, incubated at 25 °C for 48 h and 7 d, respectively. In addition, all samples were also analyzed for the presence of the main pathogenic microorganisms: coagulase-positive staphylococci (CPS) on Baird Parker (BP) supplemented with rabbit plasma fibrinogen (RPF); *Listeria* spp. and *Listeria monocytogenes* on *Listeria* selective agar base with SR0140E supplement; *Salmonella* spp. and *Escherichia coli* on Hektoen Enteric Agar (HEA). All pathogens were incubated at 37 °C for 24 h. All media and supplements were purchased from Oxoid Microbiology Products (Thermo-Scientific, Milan, Italy), except HEA provided by Microbiol Diagnostici (Uta, Italy). All plate counts were carried out in duplicate.

2.6. Sensory Analysis

The sensory analysis was performed by a panel of 10 tasters. All tasters were trained and had extensive experience in food sensory evaluation [46]. First of all the tasters were submitted to a study of the visual aspect of the fruits. After that, they were trained using different pear samples to recognise aroma, flavour and texture attributes during the training session, using product and ingredient references. All the samples were subjected to a panel consisting of 24 descriptors as follows: visual appearance (VA), flesh colour (FC), flesh lightness (FB), browning (B), pear odour (PO), herbaceous odour (HO), off-odour (OO), roughness (R), compactness (CP), dryness (D), aroma intensity (AI), crunchiness (CR), firmness (FR), stickiness (S), rubber (RU), juiciness (J), astringency (AS), sweetness (SW), bitterness (BT), acidity (AC), pear flavour (PF), herb flavour (HF), off-flavour (OF), overall assessment (OA). All samples were scored from 1 (no descriptor intensity) to 9 (highest descriptor intensity) and descriptors were evaluated from day 0 (as fresh) to day 7 (d7).

2.7. Statistical Analysis

Data were presented as mean ± standard deviation. Statistical analysis was performed using the XlStat® software version 9.0 (Addinsoft, Paris, France). Data were analysed using one-way analysis of variance (ANOVA) and Tukey's multiple range test with $p < 0.05$ considered significant.

3. Results and Discussion

3.1. Physico-Chemical Analysis

3.1.1. Weight Loss

The untreated sample (CTR) suffered a more rapid weight loss already from the second day of storage (d2), compared to the samples treated with edible coatings (Table 1). In fact, the lowest weight loss values were observed in EC2 during all the storage period. Therefore, the edible coatings (EC1 and EC2 treatments) seem to reduce juice leakage, in agreement with Seyed et al. [47], who maintained the mango cell structure longer. This positive effect in terms of weight loss reduction may be due to the hygroscopic properties of the Aloe vera gel, which is mainly composed of polysaccharides. It produces a water barrier between the fruit and the external environment [48].

Table 1. Weight loss (%) of treated (EC1 and EC2) and untreated (CTR) pear slices.

Treatments	d2	d4	d7
CTR	1.14 ± 0.03 A	1.7 ± 0.06 A	2.1 ± 0.12 A
EC1	0.8 ± 0.03 B	1.3 ± 0.05 AB	1.6 ± 0.1 AB
EC2	0.7 ± 0.03 B	1.0 ± 0.05 B	1.2 ± 0.1 B

Data corresponds to the means ± standard deviation of three replicates. Means with different letters are significantly different at $p \leq 0.05$ using Tukey's-HSD test. Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time.

3.1.2. Firmness

A progressive loss of firmness of the pear slices was observed in all the treatments during storage (Figure 1). However, in CTR samples a significantly reduction ($p < 0.05$) was registered starting from the second day (d2), in EC1 from the fourth day (d4) and in EC2 from the seventh day (d7) of storage, with final value of 2.71, 3.24 and 3.43 Kg-cm^{-2} respectively. Due to the edible coating, it seems that the pear slices kept the cell wall pectin, slowing down the cell degradation processes. During cold-storage, this behaviour could be associated with water loss and degradation of pectic substances in the pear fruit, according to Nath et al. [49] In a study conducted on Bartlett/William pear fruit, edible coatings based on polysaccharides and lipids were observed to reduce the softening of the cell structure, delaying the ripening processes due to normal enzymatic activities [50]. In fact, HPMC associated with lipid components seems to have a greater capacity to modify the internal atmosphere of the fruit and to maintain high firmness values. Moreover, aloe treatment significantly reduced the firmness losses of table grapes and papaya. This may be due to the effect of Aloe veragel on the reduction enzyme hydrolases of â-galactosidase, polygalacturonase, and pectinmethyl-esterase activities [48,51] responsible for the changes in the cell wall.

3.1.3. Flesh Colour

A progressive lightness reduction was registered in CTR samples with significative L* value reduction among all the storage days (Figure 2). A reduction in lightness values indicates a loss of gloss of the pear slices during the storage period. Indeed, the slices of the CTR sample looked dehydrated compared to the slices treated with the edible coatings.

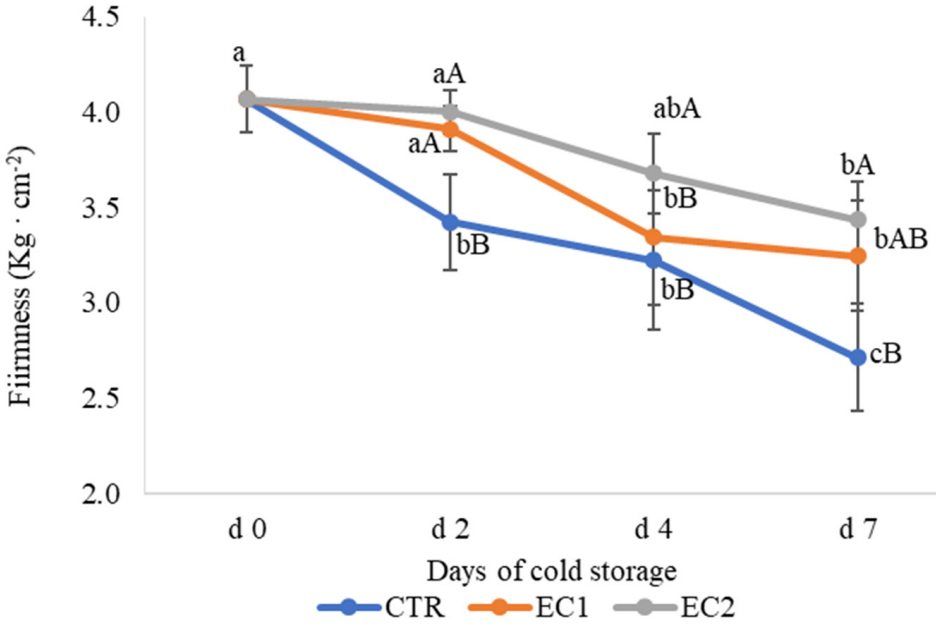

Figure 1. Development of firmness (Kg cm^{-2}) of treated (EC1 and EC2) and untreated (CTR) pear slices. Data correspond to the means ± standard deviation of three replicates. Means with different letters are significantly different at $p \leq 0.05$ using Tukey's-HSD test. Different lowercase letters denote significant differences ($p < 0.05$) between different sampling times for the same treatment. Different capital letters denote significant differences ($p < 0.05$) between different treatments for the same sampling time.

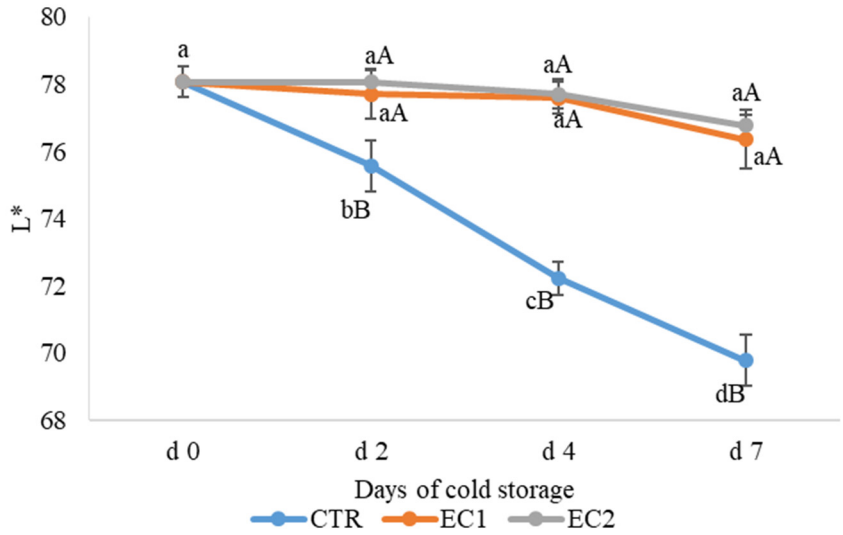

Figure 2. Development of lightness (L*) of treated (EC1 and EC2) and untreated (CTR) pear slices. Data correspond to the means ± standard deviation of three replicates. Means with different letters are significantly different at $p \leq 0.05$ using Tukey's-HSD test. Different lowercase letters denote significant differences ($p < 0.05$) between different sampling times for the same treatment. Different capital letters denote significant differences ($p < 0.05$) between different treatments for the same sampling time.

Aloe vera gel imparted an attractive natural-looking sheen to table grapes, papaya, which was correlated to lower changes in both skin color and dehydration [52,53].

However, a slight but not significative ($p < 0.05$) lightness reduction was observed in EC1- and EC2-treated samples during storage, with no significant differences between the two coating treatments. Olivas et al. [54] also reported a positive effect of combining ascor-

bic acid, calcium chloride and sorbic acid with both methylcellulose and methylcellulose-stearic acid coatings to reduces the browning of ready-to-eat 'Anjou' pears.

During the storage period, the browning index (BI) increased in CTR samples with significant differences between d2, d4 and d7 (Figure 3). Furthermore, significative but slight BI increases were registered in coated (EC1 and EC2) samples during storage while no statistical differences were observed between EC1 and EC2 at the different storage days. From the results obtained, it appears that coating treatments strongly reduced browning index values with significant differences during storage with respect to the CTR sample.

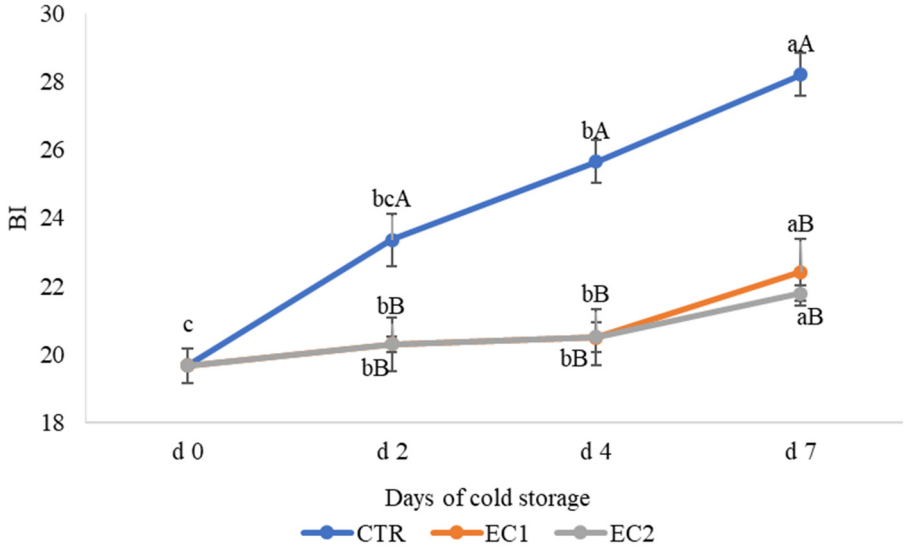

Figure 3. Development of browning index (BI) of treated (EC1 and EC2) and untreated (CTR) pear slices. Data correspond to the means ± standard deviation of three replicates. Means with different letters are significantly different at $p \leq 0.05$ using Tukey's-HSD test. Different lowercase letters denote significant differences ($p < 0.05$) between different sampling times for the same treatment. Different capital letters denote significant differences ($p < 0.05$) between different treatments for the same sampling time.

In fact, thanks to the lightness (L*) and browning index (BI), the obtained data made it possible to hypothesize an effective slowing down of the action of the polyphenol oxidase enzyme, due to the presence of the costituents of edible coating, in agreement with Sapers and Miller [55], Dong et al. [56] and Gorny et al. [57].

In fact, antioxidant constituents of the fruit, such as phenolic compounds and ascorbic acid, are linked to enzymatic browning. Phenolic compounds are oxidised to highly unstable quinones, which are then polymerised to brown, red and black pigments [58,59]. Furthermore, a decrease in ascorbic acid content below a threshold level has been correlated with the browning of the middle part of pears [60].

A similar trend was registered in terms of colour variation ΔE (Figure 4). Moreover, for this parameter, significant differences ($p < 0.05$) were observed between CTR and treated samples at the different storage days, while no statistical differences were found between EC1 and EC2 treatments.

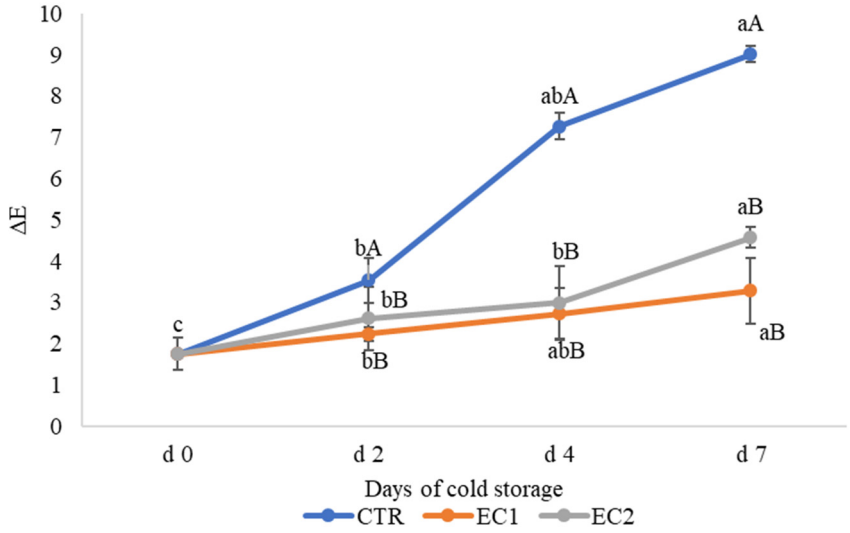

Figure 4. Development of colour variation (ΔE) of treated (EC1 and EC2) and untreated (CTR) pear slices. Data correspond to the means ± standard deviation of three replicates. Means with different letters are significantly different at $p \leq 0.05$ using Tukey's-HSD test. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment. Different capital letters denote significant differences ($p < 0.05$) between different treatments for the same sampling time.

3.1.4. Soluble Solid Content

A progressive increase in soluble solids content during storage was observed in all the treatments, with significantly higher values between d2 and d4 (Figure 5). It can be seen from that up to the second day of storage CTR and EC2 had the same values, unlike EC1 that showed SSC had significantly higher values. After that day, EC2 always remained below the CTR and EC1 values with significative lower values of SSC at d4 and d7. No statistical differences were observed between CTR and EC1 at d4 and d7.

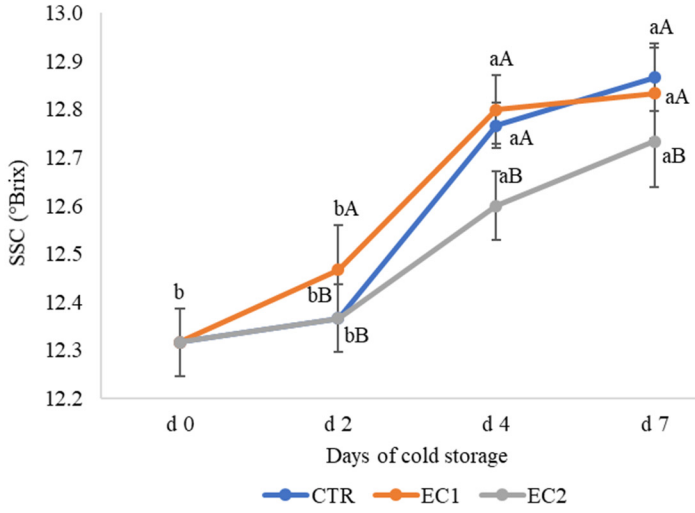

Figure 5. The changes of the soluble solids content (SSC) in °Brix, of treated (EC1 and EC2) and untreated (CTR) pear slices. Data correspond to the means ± standard deviation of three replicates. Means with different letters are significantly different at $p \leq 0.05$ using Tukey's-HSD test. Different lowercase letters denote significant differences ($p < 0.05$) between different sampling times for the same treatment. Different capital letters denote significant differences ($p < 0.05$) between different treatments for the same sampling time.

This outcome could be associated with the presence of antioxidant and anti-browning agents which, together with the Aloe vera gel, that reduced the increase in SSC due to the normal fruit ripening processes [61–64] could be associated with the presence of antioxidant and anti-browning agents which, together with the Aloe vera gel, reduced the increase in SSC due to the normal fruit ripening processes [61–64]. In addition, the increase in SSC during storage could be associated with the transformation of pectic substances, hydrolysis of starch and dehydration of fruits [65,66], leading to an increase in their concentration.

3.1.5. Microbiological Analyses

The microbiological investigation carried out by plate count throughout fresh-cut pear productions involved spoilage and pathogenic microbial groups commonly associated with fruit and vegetable items [67–69]. None of the CTR, EC1 or EC2 samples revealed the presence of detectable levels of all microbial groups that were the subject of investigation at any sampling time. The absence of microorganisms in all samples analysed is mainly due to the high contents in organic acids and the low temperatures applied during refrigerated storage [70]. Furthermore, the absence of microorganisms undoubtedly indicated the respect of the microbiological criteria for foodstuffs during the production of ready-to-eat pear fruit with and without coating.

3.1.6. Sensory Analysis

Regarding the results obtained from the panel test, including the study of visual aspects (Figure 6), the fresh fruit (d0) showed acceptable values from a commercial and consumption point of view. In particular, concerning the results obtained from the panel test, comparing the starting value (Figure 7a), it is possible to highlight the first differences between treated and untreated fruit, to d2 (Figure 7b): in particular, EC2 shows higher values in terms of firmness (FR), flavour (PF), juiciness (J) and sweetness (SW), while CTR shows a high value of browning (B) and roughness (R). At d4 (Figure 7c), all the descriptors have decreased: CTR continues to undergo browning processes and shows roughness (R) and off-odour (OO) formations, while the treatments allowed us to maintain values more similar to those of the starting fruit (d0), especially in terms of firmness (FR), juiciness (J), sweetness (SW) and pear flavour (PF). On the last day of storage (d7—Figure 7d), EC2 still shows higher values than the other treatments, although with small differences, while CTR is completely degraded, which is especially evident from the colour descriptors (FC, FB and B).

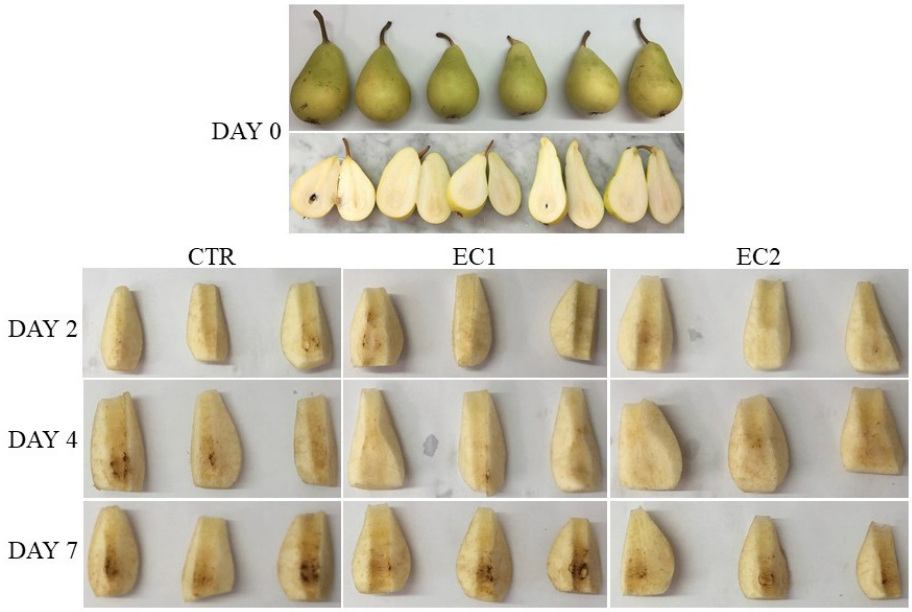

Figure 6. Visual aspect of untreated (CTR) and treated (EC1 and EC2) pear slices.

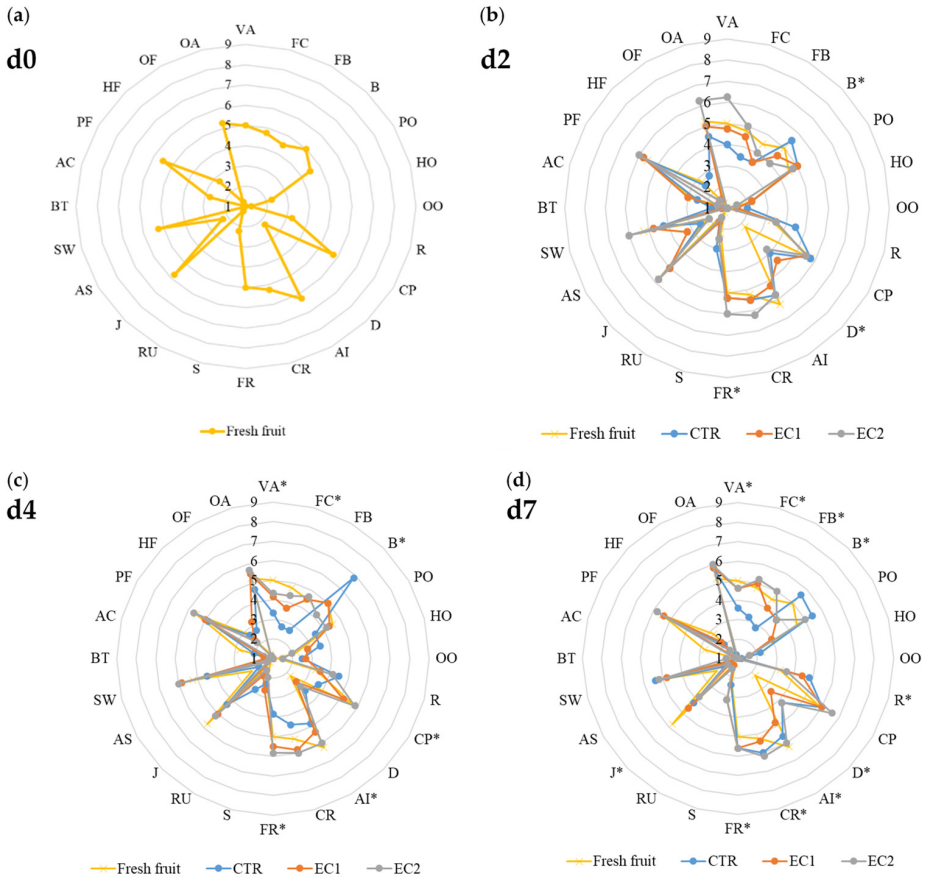

Figure 7. (**a**–**d**): Sensory analysis of treated (EC1 and EC2) and untreated (CTR) pear slices. Data correspond to the means ± standard deviation of three replicates. Data with * are significantly different at $p \leq 0.05$ using Tukey's-HSD test. Legend: visual appearance (VA), flesh colour (FC), flesh lightness (FB), browning (B), pear odour (PO), herbaceous odour (HO), off-odour (OO), roughness (R), compactness (CP), dryness (D), aroma intensity (AI), crunchiness (CR), firmness (FR), stickiness (S), rubber (RU), juiciness (J), astringency (AS), sweetness (SW), bitterness (BT), acidity (AC), pear flavour (PF), herb flavour (HF), off-flavour (OF), overall assessment (OA).

4. Conclusions

Both two edible coating maintained the physical and chemical characteristics of the fruit for 7 days and did not alter their taste. In particular, weight loss, firmness and colour indexes decrease more slowly in treated slices than in untreated while soluble solids content increase faster in untreated fruit indicating a faster ripening process. The sensory analysis showed that the tasters preferred the EC2-treated samples because of the unaltered flavours until the last day of storage. However, the presence of PSO in the EC1 did not show significant differences in terms of firmness and weight loss, compared to the EC2 treatment, but it might be useful to study its effects during a longer storage period and in different concentration to highlight their positive effect. In fact, in consideration of its properties of functional food, future studies of nutraceutical traits of fresh cut pear treated with PSO-based coating will be conducted.

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