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Evaluation of Aloe arborescens gel as new coating to maintain the organoleptic and functional properties of strawberry (Fragaria × ananassa cv. Cadonga) fruits

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Original

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Evaluation of Aloe arborescens gel as new coating to maintain the organoleptic and functional properties of strawberries (Fragaria x ananassa cv. Cadonga) fruits

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- 3 Evaluation of *Aloe arborescens* gel as new coating to maintain the organoleptic and functional
- 4 properties of strawberries (Fragaria x ananassa cv. Cadonga) fruits
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Summary

- Strawberries (*Fragaria x ananassa* ev. Cadonga) are highly perishable fruits with a storage life, which may be less than a week. In this study, *Aloe arborescens* gel was used as postharvest treatment in order to maintain strawberry quality. Strawberries coated with edible *A. arborescens* gel were packaged in a polypropylene box and stored. Fruit titratable acidity, pH, soluble solid content, ascorbic acid, total phenols, total flavonoids, total anthocyanins and antioxidant activity evaluated by two different tests (DPPH and ABTS) were measured during 14 days of storage. Significant differences were found (p< 0.05) for the samples treated with *A. arborescens* compared to the control. During conservation, use of *Aloe* gel maintained lower values for total soluble solids, a higher concentration of total phenols and ascorbic acid, and a better antioxidant activity when compared to the control.
- **Keywords:** Strawberry; Edible coating; *Aloe arborescens* gel; Postharvest quality.

The anthocyanin content remained largely unchanged throughout, in all compared samples.

Introduction

The deterioration of fruits after harvesting are mainly due to the interactions between the fruits and its surroundings, which can result in loss of moisture and of some compounds (Andrade Pizarro et al., 2016). The strawberry (Fragaria × ananassa) is a shrub belonging to the Rosaceae family (Kim et al., 2011) The fruit is soft and can be consumed fresh or processed in puree, juice or jam. These fruits are a low-calorie (36 calories × 100 g) source of bioactive compounds including polyphenols and vitamin C (Soares, 20014). Strawberries have a very short shelf-life due both to the high respiration rate and to the absence of peel or rind and, therefore, strawberries rapidly lose water and weight (Pelayo et al., 2003; Parvez & Wani, 2018). For this reason, fresh, refrigerated fruits should be consumed within 2-3 days after manual picking. Strawberries are usually packed on-site in plastic punnets with lids.

To lengthen the shelf-life of fresh fruit, a correct choice of packaging must be taken into account (Sicari et al., 2017; Rizzo et al., 2018; Giuffrè et al., 2019). Today, there are a variety of packaging methods, some of which use innovative materials and techniques. A common aspect of modern packaging is to offer "convenience" and ease of use, combined with a minimal environmental impact (Sicari et al., 2017; Rizzo et al., 2018; Giuffrè et al., 2019). In recent years, research has been directed towards edible coatings, which can fulfil many of the above-mentioned requirements. Edible coatings are materials, which mimic the natural external layer of fruit and vegetables (Del Valle et al., 2005; Chien et al., 2007; Tzoumaki et al., 2009). These coatings have many advantages: they are biodegradable materials that can be eaten together with the produce, and reduce the environmental impact by wholly, or in part, replacing synthetic packaging with particular reference to plastics. These coatings provide a barrier to moisture and oxygen, and consequently they reduce weight loss during the fruit's shelf-life (Akhtara et al., 2015). The application of edible coating can be carried out by brushing, spraying or dipping (Mchugh & Senesi, 2000). Furthermore, to edible coatings additives may be added, especially antimicrobial, to help extend the shelf-life of the produce, as has been

widely reported in the literature (Krasaekoopt & Mabumrung, 2008; Rojas-Graü et al., 2008). Furthermore, several studies propose the addition of texture enhancers to edible coatings to minimize softening during storage of fresh-picked fruits (Rojas-Graü et al., 2008). Recently, Aloe vera gel has gained much attention for use as an environmentally friendly and safe post-harvest treatment. It has been proposed as a coating for fresh fruit since it does not change the food's taste and appearance (Misir et al. 2014) and can be used as a completely normal and useful alternative to artificial preservatives. Valverde, et al. (2005) and Martínez-Romero et al. (2006), used it as a coating for cherries and table grapes, and it was shown not only to be useful in reducing microbial proliferation, but also to have a positive effect on reducing moisture loss, on maintaining firmness, and in checking respiration. A similar effect was observed also for peach, nectarine, guava, plum and kiwi fruits (Guillén et al., 2013; Hazrati et al., 2017). More recently apart from A. vera gel, the gels obtained from other *Aloe* spp. such as *A. arborescens* and *A. ferox* have been investigated for this purpose. Taking into account the increased consumer interest in ready-to-eat fruit with high nutritional value, we wished to evaluate the protective effect of the application of *Aloe arboresces* gel as edible coating on strawberry fruits. The effectiveness of A. arborescens gel, used as an edible coating, was evaluated and compared to a widely used, commercially available, polypropylene-based anti-fog film (PP). Untreated and unpackaged strawberries were used as a control.

Materials and methods

Chemicals and Materials

Folin-Ciocalteu reagent, 2,2-diphenil-1-picrylhydrazyl radical (DPPH*+) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS*+), were supplied by Carlo Erba (Milan, Italy). Solvents and reagents not expressly specified had a high degree of purity and were supplied by Carlo Erba (Milan, Italy), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and aluminium chloride (AlCl₃), were supplied by Aldrich (Milan, Italy). Anti-fog film (PP). was supplied by Cartonpack, Rutigliano, BA, Italy.

Preparation of *Aloe arborescens* gel

Aloe arborescens leaves used for the production of the gel were harvested from plants, approximately 6 years old, growing wild in the province of Reggio Calabria (Italy). The leaves chosen were neither the old leaves from the bottom of the plant, nor the new leaves from the top, but rather were chosen from the middle part of the plant. The fresh leaves were picked in the early hours of the morning and immediately taken to the Food Technology laboratory, where they were washed with 2% v/v chlorinated water (sodium hypochlorite). After drying, to extract the gel, the parenchyma was ground in a commercial blender. The obtained matrix was filtered to remove the fibrous fraction, under vacuum filtration with Buchner funnel using laboratory filter paper. The gel was pasteurized in a thermostatic bath at 70 °C (core) for 45 minutes (Arowora et al., 2013), and allowed to cool immediately to room temperature before further use. The treatment was monitored using a Data Logger (Escort Junior, Astori tecnica, Italy). After pasteurization and cooling, the gel was stored in brown glass bottles to prevent oxidization.

Fruit selection and treatments

Fragaria x ananassa ev. Cadonga fruits were manually picked in the early hours of the morning in May 2017 from a farm in the province of Reggio Calabria (Italy). They were harvested at a commercially mature stage, sorted to eliminate damaged, shrivelled, and unripe fruit, and selected for uniform size and color. Following this screening, the berries were washed with chlorinated water (sodium hypochlorite 2% v/v), rinsed with distilled water, carefully dried with paper towels, and placed in plastic punnets (15 cm x 6 cm x 6 cm), each of them containing around 250 g of strawberries (about eighteen fruits). Forty-two of these containers were filled, of which fourteen samples had no treatment other than being washed in chlorinated water (C, control), fourteen sample containers were sealed using a commercially-available polypropylene-based anti-fog film (PP), and fourteen samples contained fruit treated with Aloe arborescens gel (EC). The treatment with EC was carried out by immersing fruits for 1 min at room temperature in Aloe arborescens gel. Three replicates for each treatment were conducted. Based on our previous studies, longer dipping times showed no change in results (data not shown). After dipping, the fruits were placed on stainless steel trays and air-dried for 60 minutes at 15 °C. All forty-two samples, subdivided as described, were refrigerated at 3 ± 1 °C (90 % RH).

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Monitoring of shelf-life parameters

Changes in the shelf-life parameters such as weight loss, whole-fruit firmness, were monitored at time 0, 2, 5, 7, 9, 12, and 14 days of storage. All the analyses were carried out in triplicate.

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Weight loss measurement

To determine weight loss, strawberries were weighed by using Analytical Balance ML54T/00 (Mettler Toledo S.p.A. Milan, Italy) at the beginning of the experiment and after coating (t=0), and thereafter each sampling during the storage period. Weight loss was expressed as the percentage loss of the initial total weight. Ten fruits in three repetitions were used to evaluate the weight loss.

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Fruit colour surface measurement

The strawberries' surface colour was measured at room temperature using Konica Minolta CM-700/600d spectrophotometer (Konica Minolta Sensing, Inc., Japan). CIE L* a* b* parameters were recorded. The values a^* and b^* were used to calculate the Chroma value (C) using the following equation: $C = \sqrt{a^{*2} + b^{*2}}$

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Whole-fruit firmness measurement

Fruit firmness was measured as total firmness by a compression test. TA.XT PLUS Texture Analyser (Stable Micro Systems, Godalming, UK) was used, which profiled a mechanical force displacement using a 50 kg loading cell and equipped with a 5 mm diameter flat probe. Fruit firmness values

were an average of 6 strawberries. Each sample was subjected to a two-cycle compression with 5 s between cycles. The highest value of force required to compress the sample during the first compression cycle was recorded as fruit firmness. This test measured individual fruit firmness based on the resistance of the flesh to deformation by the probe. Firmness was measured at the equatorial part and on two faces of each fruit. Three repeated measurements were performed for each sample and the results were expressed in Newton (N).

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Determination of pH, titratable acidity, total soluble solid and ascorbic acid content

Strawberries (c.a. 5 g) were ground in a commercial blender, 50 mL of H₂O was added before homogenization using Ultraturrax T-25 (Ika Labortechnik, Janche & Kunkel, Milan, Italy). The mix was centrifuged at 5000 rpm for 10 min and the supernatant was collected and used for all determinations.

A Crison basic 20 pH meter (Crison Instruments S.A., Milan, Italy) was used to test the pH of strawberry samples. Titratable acidity (TA) results were expressed as citric acid percentage on dryweight basis. Strawberry soluble solid content (TSS) was measured at 20 °C using a digital Atago Model PR-101 α refractometer (Atago Co. Ltd, Milan, Italy). Results were reported as Brix degrees (°Brix). All determinations above described were carried out in triplicate. The concentration of ascorbic acid in strawberries was measured by the method reported by Thimmaiah, (1999). Results were expressed as mg of ascorbic acid/100 g of fresh weight (FW).

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Strawberry extraction procedure

Fresh strawberries (10 g) were homogenized (1 min at 24000 rpm) with 30 mL of methanol/water/hydrochloric acid (80:19.9:0.1, % v/v) solution using Ultraturrax T-25 homogeniser (Ika Labortechnik, Janche & Kunkel, Milan, Italy), and further extracted at room temperature under continuous stirring for 1 h in the dark. The residue obtained by vacuum-filtration (Whatman n. 1 fiter, Vetrotecnica Srl, Padova, Italy) was re-extracted three times (until colourless) under the same

conditions to maximize the antioxidant recovery. The filtrates were combined, evaporated to dryness using a rotary evaporator, and dissolved in a methanol/water mixture for further analysis.

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Evaluation of total phenols and flavonoids content

weight (FW). All analyses were carried out in triplicate.

The total polyphenol content (TPC) of Strawberry fruit was determined by the Folin-Ciocalteu method as previously reported (Singleton et al., 1999). The absorbance was measured at $\lambda = 760$ nm. The results were expressed as gallic acid equivalents (GAE) in mg/100 g FW. The total flavonoid content (TFC) in strawberry extract was measured by the aluminium chloride colourimetric assay as previously reported (Zhishen et al., 1999). The absorbance was measured at 510 nm. The total flavonoid content was expressed as mg catechin equivalents (CE)/100 g of fresh

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Determination of total anthocyanins

Total anthocyanin content (TAC) was measured using the described method (Kara & Ercelebi, 2013).

Absorbance was measured at 510 nm and 700 nm in buffers at pH 1.0 and pH 4.5. Experiments were

conducted in triplicate.

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Evaluation of antioxidant activity

The antioxidant activity (AA) was screened by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods.

The DPPH test was assessed following the method reported by Brand-Williams et al. (1995). Briefly,

DPPH· methanolic solution was added to strawberry extract. The absorbance at λ = 515 nm was

measured. All tests were carried out in triplicate and the results expressed as means ± standard

deviation (SD).

The ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonate) radical test was carried out as

described by Re et al. (1999). Briefly, a solution of ABTS radical was diluted (1:80) with ethanol to

give an absorbance of 0.70 at $\lambda = 734$ nm. An aliquot of extract was added to ABTS solution. Trolox was used as a standard antioxidant and fruit activity was expressed in mM of Trolox equivalents.

Statistical analysis

Results were expressed as mean \pm SD of three replicates. All data were analyzed using one-way analysis of variance (ANOVA) with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) statistical software. Significant differences were calculated according to Duncan's multiple range tests. Differences at P<0.05 were considered to be statistically significant while at P<0.01 were considered to be highly significant. PCA was performed on the auto-scaled data matrix, and the principal components were extracted so that the dimensionality of the original data matrix was reduced while retaining the maximum variability. SPSS software was used for statistical analysis of PCA.

Results and discussion

Weight loss

Since strawberries have no peel or waxy substance on the skin, these fruits are susceptible to rapid moisture evaporation, resulting in softening, shrinking, surface wounding, and darkening of skin colour. The use of edible coatings such as *A. arborescens* gel makes a layer on strawberry skins that reduces water loss, defends against mechanical damage and microbial attack, and also seals small wounds. The loss of strawberry weight during the shelf-life period represents not only a quality factor, but also a problem of economic impact for producing companies. As expected, the control sample shows the greatest weight loss (13.52%), whereas the samples treated with *A. arborescens* gel (EC) and polypropylene-based film (PP) show a loss of 1.16% and 0.19% respectively. The analysis of variance shows that the percentage of weight loss for strawberries coated with *Aloe arborescens* gel was significant (p<0.01) compared to the control. These results are in agreement with those of Mahmoud & Savello, (1992) and Avena-Bustillos *et al.* (1997) who concluded that coatings and/or films significantly conserved water content. Even if the samples in the polypropylene-based film (PP)

show less weight loss (data not shown), it can be hypothesized that the aloe gel coating nonetheless was effective in reducing weight loss during refrigerated storage. This reduction in weight loss on the part of the strawberries coated with aloe gel is due to the gel's acting as a barrier to the movement of moisture, thus reducing its loss during post-harvest storage. Our results are better than those obtained by Singh et al. (2011), who used A. vera gel as edible coating on strawberries stored for 16 days and obtained a weight loss of 9.99 % compared to the untreated fruit (13.79%). The superiority of A. arborescens gel compared to A. vera gel is also demonstrated. In fact, Nasrin et al (2017) reported a weight loss of 10.67% in control and 3.68% in treated fruits after nine days of storage.

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Fruit firmness

The fruit firmness, for all treatments, followed a declining trend commensurate with length of storage. However, PP and EC significantly maintained firmness compared with control (PP: 2.91 and EC: 2.72 vs C: 1.83 after 9 days of storage). Interestingly, the firmness value is 3.3-time higher in EC sample in comparison to the control at the end of the observation period. The analysis of variance shows that strawberry firmness for strawberries coated with Aloe arborescens gel was significant (p<0.01) compared to the control. Recently, Nasrin et al. (2017) found that strawberries treated with A. vera gel lost only 18.43% of firmness in comparison to uncoated fruits after 15 days of storage. We can state that edible coating showed a good result for firmness probably because this coating slowed down metabolism. These results were in agreement with those of Maftoonazad & Ramaswamy, (2005) and Koh & Melton, (2002), who stated that retention of firmness could be explained by retarded degradation of insoluble protopectins to the more soluble pectic acid and pectin.

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Physical chemical parameters

Table 1 shows the values of the analyses carried out on the strawberries in three different storage conditions (C, PP, EC). Titratable acidity (TA), total soluble solids (TSS), pH and colour were monitored over the 14 days of storage at 3 ± 1 °C. The untreated samples (C) had a pH of 3.45 when

freshly picked (t=0). The addition of A. arborescens gel (EC) or storage under film (PP) did not cause significant differences to pH when compared to the control (C). Generally, no significant modification in pH was observed during the period of observation in all investigated samples. There were significant differences for AT, TSS and colour among different treatments during the storage of 14 days (Table 1). The effects of coating treatments on the TA and TSS parameters during storage are shown in Table 1. The value for titratable acidity was 0.49 g/L at t=0. This value increased for all three storage types (C, PP, EC) although the greatest increase was in the control sample at t=14, which had a value of 0.98 g/L, whereas the samples stored using *Aloe arborescens* gel or stored in PP film had very similar values (0.74 and 0.71 g/L respectively). The TA levels in the control and coated samples gradually decreased during the storage period, and the difference was significant in the control sample only on day 9. However, the decreasing trends of TA in coated samples were not significant during the storage period. These results are in disagreement with those reported by Vahdat et al. (2010), that found a high TA value in A. vera gel treated strawberries in comparison with untreated fruits. The TSS of the control, coated gel and PP film significantly increased with storage time, while the coated and PP film samples showed a slight increase compared to the control sample (Tab. 1). In fact, at t=0 strawberries showed a total soluble solids (TSS) value of 7.20 °Brix. Fruits treated with A. arborescens gel or film (PP) maintained a value of 8.20 °Brix, whereas, for the control, the value of TTS reached 11.20 °Brix at t=14, similar to the values reported by Benítez et al., 2013. This increase in TSS could be due to the hydrolysis of structure polysaccharides, and the release of simple compounds (sugars). This hypothesis could be corroborated by the loss of consistency, due to the hydrolysis of the same polymers. However, these data are in disagreement with those reported by Nasrin et al. (2017) and Sing et al. (2011), who found a moderate increase of TSS content in A. vera gel treated fruits. The use of Aloe gel as a coating does not seem to influence L^* parameter. For

Chroma, the three sets of values show a similar trend for the first 7 days of storage while a drastic

reduction of C value was observed after 12 days of storage in all samples, although the sample treated

with EC presents a lower reduction of Chroma compared to PP and C (Table 1). This reduction in chromatic coordinates correspond to a dark red color on the skin of the fruit as commonly happens when strawberries become fully ripe. The application of *A. arborescens* gel coating maintains the fruit's colour intensity, whereas the control or the fruit in polypropylene-based film show a more marked change of colour. Certainly, the absence of any protection for the control fruit accelerated the fruit's post-harvest metabolic aging. The data obtained are similar to those in the literature regarding the use of different edible coatings for strawberries. In particular, our data are in agreement with those for strawberries treated with *A. vera* gel (Ribeiro *et al.*, 2017). A reduction in C and L* values was observed for combined chitosan-oleic acid edible coated strawberries refrigerated at 5 °C for 10 days (Vargas *et al.*, 2006).

Bioactive compound content

Epidemiological studies showed that strawberry consumption has been associated with several health benefits probably due the antioxidant activity of its bioactive constituents (Forbes-Hernández *et al.*, 2016; Giampieri *et al.*, 2017). For this purpose we decided to monitor the content of bioactive constituents including ascorbic acid content (AC), total polyphenol content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC) during strawberry shelf-life. Results are reported in Table 2. Sapei & Hwa, (2014) found that freshly-picked strawberries have a high and extremely variable ascorbic acid content, usually between 5 and 50 mg/100 g of FW. At t=0 we found the acorbic acid content was 56.37 mg/100 g, reaching at t=14 values of 56.39, 65.79, and 74.59 mg/100 g for C, PP and EC respectively. The analysis of variance shows that *Aloe arborescens* gel coating and PP film on ascorbic acid of strawberry (Tab. 2) were significant (p<0.01) compared to the uncoated fruit.

As reported in the literature (Weichmann *et al.* 1985; Salunkhe et al., 1991), the oxidation of vitamin C is carried out by ascorbic oxidase and phenoloxidase, which have a low affinity for oxygen. Ascorbic acid content decreased during storage for uncoated strawberries (C). *Aloe arborescens* gel coating was effective in reducing the ascorbic acid loss (Tab. 2). The application of *Aloe* gel coating

preserved AA content, and our results are in line with those reported by Singh et al. (2011) and Nasrin et al. (2017) however both these studies used A. vera gel as fruit coatings. The total phenolic compounds were affected by the treatments and the storage time. In Table 2 it can be seen that until t=2 total polyphenol values do not show significant variations (p<0.01) for all three treatments (C, PP, EC), whereas from t=5 the total polyphenol content in C differs significantly from PP and EC. At t=0 the strawberries showed a TPC value of 97.83 mg/100 g FW. During storage, the TPC in samples C and PP increased to t=5 then decreased to t=14 giving a final value of 68.14 and 85.62 mg/100 g FW for samples C and PP respectively. For those strawberries treated with A. arborescens gel (EC) the TPC increased to t=5 (112.52 mg/100g) and t=7 (115.24 mg/100g), before slowly decreasing. This may be due to the continued biosynthesis of the compounds after harvesting. However, unlike samples C and PP, the final TPC for EC was very close to the t=0 value. As opposed to what was found for total polyphenols and vitamin C, the total anthocyanin content showed a slight increase in the control sample, from 18.54 mg/100 g FW at t=0 to 18.22 mg/100 g FW at t=14 days, peaking at 24.23 and 24.12 mg/100 g FW at t=5 and t=7, respectively. For the sample treated with aloe gel, however, total anthocyanins increased to 21.41 mg/100 g at t=14 days. The anthocyanin of treated fruit increased during cold storage, similar to those reported previously (Hassanpour, 2015), that may be due to the continued biosynthesis of the compounds after harvesting. This trend can be explained by the barrier effect of the gel, which modifies the fruit's metabolism by reducing respiration (Martínez-Romero et al., 2006). Flavonoids followed a similar pattern with total phenolics, having higher values after 7 days shelf life (Table 2). Total flavonoid content at t=0 was 236.22 mg/100 g FW. Over the first five days, a sharp increase was observed, especially in strawberries coated with A. arborescens gel. At t=14, samples C and EC showed a significant increase with highest content in EC (652.96 mg/100 g FW) whereas strawberries in polypropylene-based film (PP) showed a smaller increase, although still greater than at t=0. Probably because this coating reduces metabolism.

Antioxidant activity

Table 2 reports the results of the antioxidant capacity of strawberry extracts by DPPH and ABTS assays. Obtained data, expressed in mM Trolox equivalents, show that EC samples, during the experiment, have higher antioxidant activity compared to the others (C and PP). In particular, at t=14 days the antioxidant capacity of EC strawberry extract was 0.92 mM Trolox for DPPH test and 8.46 mM Trolox for ABTS assay. From our results, it is possible to see how the samples treated with *A. arborescens* gel show a much greater content of polyphenols and flavonoids compared to samples C and PP. Similar results have been found by Gol *et al.*, (2013). Therefore, we can say that the coating reduces damage to the strawberries, stimulating a scavenging activity towards the radicals DPPH and ABTS.

Principal Component Analysis

Results were analyzed by a multivariate PCA method to reach a smaller number of artificial variables accounting for most of the variance in the observed variables (D'Agostino *et al.*, 2014).

The obtained results are shown in Fig. 1. In all considered samples, the first five PC accounted for 100% of total variance. Two PC's (PC1 and PC2), explaining the cumulative 80% of the data variance, were chosen based on the Eigen values (> 1). The Scree plot shows the variance of each component in the dataset, used to determine how many components should be retained in order to explain a high percentage of the variation in the data. As can be seen in Fig. 1 most of the variance in strawberries are explained by PC1 and PC2. PC1 explains about 45% and PC2 about 35% of the total variance. Fig. 1 showed that the values of the analyses carried out at sampling t=12 and t=14 are very close to each other and are characterized by a high value in component 2. The (t=0) showing negative values on PC1 and PC2, while (t=2, t=5, t=7 and t=9), show positive values on PC1 and PC2. In the analysis of the loading variable, a correspondence clearly appeared between PC1 and TPC (C, EC and PP), ABTS (PP), Chroma (C, PP, EC), AA (C), TAC (C) and AC (PP).

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In particular, the analysis of the loading variables clearly showed the positive correlation of PC2 with AC (C and EC), TFC (C, PP and EC), AA (EC and PP), ABTS (PP), Brix (C and PP). Negative correlations were observed on PC1 and PC2 with DPPH (EC and PP), ABTS (C), and pH (EC and PP).

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Conclusions

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The application of bio-based edible coatings to prolong the shelf-life of fruits, which are in line with sustainable agriculture practices, is, at present, a hot research topic. Aloe gel coating has shown itself 346 to be a promising method of conservation since it does not affect taste and appearance of the fruits.

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In this paper, we propose, for the first time the application of *Aloe arborescens* gel to fruits. Results showed that A. arborescens gel coatings may be an important way to conserve strawberries during

refrigerated storage, and may be used instead of the plastic films, which are commercially available

today. The species A. arborescens has been shown to be better than A. vera gel for this application.

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Therefore, this type of edible coating could become a useful treatment to improve quality and

lengthen shelf-life of these widely-consumed fruits. However, further studies are necessary for the

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commercial application of A. arborescens gel in order to test different concentrations of gel find the

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optimum gel concentration for post-harvest treatments.

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Abbreviations

AA, Ascorbic acid content; ABTS, 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; C,

Control; **DPPH**, 1,1-Diphenyl-2-picryl-hydrazil; **EC**, Edible Coating with *Aloe arborescens* gel; **N**,

Newton; **PP**, Polypropylene film; **TA**, Titratable acidity; **TAC**, Total Anthocyanins Content; **TFC**,

Total Flavonoids Content; **TSS**, Total soluble solid content; **TPC**, Total Polyphenols Content.

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Conflicts of interest

Authors declare no conflict of interest.

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Table 1 Change in strawberry fruit titratable acidity (TA), pH, total soluble solid content (TSS) during storage at 3°C, in control, PP film and *Aloe arborescens* coated.

Analysis	Shelf-life monitoring period (days)									
		0	2	5	7	9	12	14		
TA										
	С	0.49 ± 0.02^{aE}	0.74±0.03 ^{bC}	0.67±0.01 ^{cD}	0.98±0.06 ^{aA}	0.88±0.03 ^{aB}	0.91±0.04 ^{aB}	0.98±0.04 ^{aA}		
	PP	0.49 ± 0.02^{aE}	0.84±0.0 ^{1aA}	0.81±0.02 ^{aB}	0.81±0.05 ^{bB}	0.67±0.02 ^{cD}	0.65±0.03 ^{cD}	0.71±0.06 ^{bC}		
	EC	0.49 ± 0.02^{aD}	0.81±0.03 ^{aB}	0.72±0.01 ^{bC}	0.95±0.04 ^{aA}	0.74±0.03 ^{bC}	0.74 ± 0.02^{bC}	0.74±0.03 ^{bC}		
рН										
	C	3.45 ± 0.10^{aC}	3.29 ± 0.02^{bD}	3.42 ± 0.22^{aC}	3.13 ± 0.17^{aE}	3.30 ± 0.24^{aE}	3.75 ± 0.12^{aA}	3.58±0.27aA		
	PP	3.45±0.11 ^{aB}	3.24±0.03 ^{bC}	3.33±0.16 ^{bC}	3.02±0.21 ^{aD}	3.35±0.28 ^{aC}	3.51±0.17 ^{bA}	3.56±0.33aA		
	EC	3.45±0.11 ^{aAB}	3.52±0.2 ^{aA}	3.33±0.18bBC	3.04±0.26 ^{aD}	3.31±0.16 ^{aD}	3.46±0.16 ^{bAB}	3.61±0.41aA		
TSS										
	С	7.20±0.14aE	7.22±0.55aE	8.11±0.33 ^{aD}	9.10±0.22 ^{aC}	11.25±0.88aA	10.10±0.88aB	11.20±0.88aA		
	PP	7.20±0.2 ^{aD}	6.25±0.3aF	7.12±0.52 ^{aE}	7.10±0.62aE	9.20±0.41 ^{aA}	8.10±0.75 ^{aC}	8.20±0.96aB		
	EC	7.20 ± 0.16^{aD}	7.30±0.41 ^{aC}	6.20±0.31aF	6.20 ± 0.55^{aF}	7.10±0.62aE	8.10±0.67 ^{aB}	8.20±0.74aA		
Color										
	С	$17,2\pm0.32^{aD}$	16,62±0.56aE	21,12±0.87 ^{bC}	23,64±1.65dA	21,36±0.87fB	8,83±0.58 ^{fF}	8.52±0.58bG		
Chroma	PP	17,2±0.31aE	$23,84\pm0.57^{aB}$	20,51±0.69bD	23,58±1.54eC	27,79±1.06cA	13,12±0.87eF	12.88±0.69b0		
	EC	$17,2\pm0.32^{aE}$	$22,12\pm1.03^{aD}$	23,19±0.67abC	23,34±1.06fB	24,03±1.12eA	$13,73\pm0.96^{dF}$	13.45±1.03b0		
L*	С	27,54±0.78aA	25,42±0.98aA	31,56±1.21 ^{aA}	27,98±2.05 ^{aA}	29,21±1.87bA	30,24±2.44bA	29.36±3.01aA		
	PP	27,54±0.74 ^{aAB}	26,52±0.89aB	27,85±1.36 ^{aAB}	27,63±2.22 ^{bAB}	31,53±1.48 ^{aAB}	32,75±2.36 ^{aB}	31.02±2.87 ^{aA}		
	EC	27,54±0.76 ^{aD}	27,03±1.03 ^{aE}	26,15±2.04abG	26,59±2.31cF	27,67±1.89 ^{dC}	29,74±3.65 ^{cA}	28.84±2.13al		

Abbreviations: C: control; PP: polypropylene-based anti-fog film; EC: Edible coating; TA: titratable acidity; TSS: soluble solid content. Data are reported as mean \pm standard deviation (n=3). Differences were evaluated by one-way analysis of variance (ANOVA) test completed with a multicomparison Tukey's test. ** p < 0.05 compared with the positive control. Means in the same row with different capital letters differ significantly (p < 0.05), means in the same column with different small letters differ significantly (p < 0.05).

Table 2 Change in strawberry fruit total phenols (TPC), total flavonoids (TFC), total anthocyanin (TAC) ascorbic acid (AA) and oxygen radical absorbance capacity during storage at 3°C, in control, PP film and *Aloe arborescens* coated.

Analysis		Shelf-life monitoring period (days)							
-		0	2	5	7	9	12	14	
TPC									
GAE mg/100 g FW	С	97.83±3.21 ^{aB}	105.24±4.33 ^{aA}	108.03±5.36 ^{bA}	96.25 ± 2.44^{cB}	88.24±3.02°C	77.51 ± 4.33^{cD}	68.14±3.55 ^{cE}	
	PP	97.83±2.17 ^{aC}	102.44 ± 4.67^{aB}	110.56±5.01abA	105.36±5.39bB	100.21±3.54bC	89.68±4.21 ^{bD}	85.62±4.33 ^{bD}	
	EC	97.83 ± 2.66^{aD}	101.28±3.88 ^{aC}	112.52±4.87 ^{aA}	115.24±6.31 ^{aA}	104.36 ± 2.88^{aB}	98.51 ± 4.09^{aD}	94.36±4.30 ^{aD}	
TFC									
CE mg/100g FW	С	236.22±3.56 ^{aF}	532.31±5.34 ^{cE}	664.32±2.98 ^{bC}	718.53±6.26 ^{bA}	690.40±4.22bB	667.97±3.11 ^{aC}	636.34±4.22 ^{bD}	
	PP	236.22 ± 3.85^{aF}	573.26±5.62 ^{bB}	659.97±4.23 ^{bB}	662.50 ± 6.07^{cB}	783.42±5.09 ^{aA}	532.32±2.69 ^{cD}	477.60±3.44 ^{cE}	
	EC	236.22 ± 4.09^{aE}	629.50±6.22 ^{aD}	794.83±3.67 ^{aA}	778.65 ± 5.88^{aB}	645.30±5.22°C	625.58±4.36 ^{bD}	652.96±5.22 ^{aC}	
TAC									
CYE mg/100g FW	С	18.54 ± 0.88^{aD}	22.14±0.77 ^{aB}	24.23±0.99 ^{aA}	24.12 ± 1.36^{aA}	21.24 ± 1.67^{aC}	20.36 ± 1.66^{aC}	18.22 ± 2.01^{cD}	
	PP	18.54±1.23 ^{aC}	20.12±1.36 ^{bABC}	22.14±1.08bA	21.41±1.57 ^{bAB}	22.01±2.09aAB	21.98±2.07 ^{aA}	$20.87\pm2.33^{\text{bBC}}$	
	EC	18.54 ± 1.37^{aB}	18.67±1.64cB	19.21±1.22cB	18.54 ± 1.6^{4cB}	19.45±2.66bB	19.99±2.03aAB	21.41±2.38 ^{aA}	
AA									
mg/g FW	C	56.37 ± 2.56^{aB}	82.72 ± 3.26^{aAB}	78.15±4.33bA	63.64±2.64 ^{cAB}	74.63±3.45cAB	69.34±3.54 ^{bAB}	56.39±4.21cA	
	PP	56.37 ± 3.74^{aF}	73.53 ± 4.21^{bC}	74.07±5.02°C	81.82±2.09bB	83.96 ± 6.23^{bA}	63.87±3.19cE	65.79 ± 4.39^{bD}	
	EC	$56.37 \pm 3.69 aF$	70.85 ± 3.89^{cE}	88.89±4.77 ^{aA}	87.27±3.01aB	85.30±4.21aC	82.98 ± 4.01^{aD}	84.59 ± 3.85^{aC}	
DPPH									
(mM Trolox)	C	1.37 ± 0.02^{aA}	0.79 ± 0.03^{cC}	0.88 ± 0.01^{bB}	0.79 ± 0.02^{cC}	0.74 ± 0.01 ^{cD}	0.78 ± 0.02^{bC}	0.67 ± 0.01^{cE}	
	PP	1.37 ± 0.01^{aA}	0.84 ± 0.02^{bC}	0.87 ± 0.01^{bB}	0.83 ± 0.02^{bC}	0.78 ± 0.01^{bD}	0.72 ± 0.01^{cE}	0.83 ± 0.02^{bC}	
	EC	1.37±0.02 ^{aA}	0.94 ± 0.02^{aC}	0.89 ± 0.02^{aE}	0.88±0.03aE	0.93±0.02 ^{aC}	0.97 ± 0.01^{aB}	0.92 ± 0.01^{aC}	
ABTS						•			
(mM Trolox)	С	8.10±0.65 ^{aA}	7.42 ± 0.44^{cD}	7.62 ± 0.77^{bC}	7.09 ± 0.88^{cF}	7.21 ± 0.88^{cE}	7.89 ± 0.61^{bB}	7.51 ± 0.44^{cD}	
	PP	8.10 ± 0.56^{aC}	8.23±0.36 ^{aB}	8.09 ± 0.65^{aC}	8.29 ± 0.74^{bC}	8.44 ± 0.49^{bA}	7.87 ± 0.59^{bD}	7.77 ± 0.82^{bE}	
	EC	8.10±0.38 ^{aE}	8.14 ± 0.29^{bE}	7.59 ± 0.56^{bF}	8.64 ± 0.6^{2aC}	9.23±0.61 ^{aA}	9.08 ± 0.67^{aB}	8.46 ± 0.93^{aD}	

Abbreviations: C: control; PP: polypropylene-based anti-fog film; EC: Edible coating; TPC: total phenols content; TAC: total flavonoids content; TAC: total anthocyanins content; AA: vitamin C/ascorbic acid content. Data are reported as mean \pm standard deviation (n=3). Differences were evaluated by one-way analysis of variance (ANOVA) test completed with a multicomparison Tukey's test. ** p < 0.05 compared with the positive control. Means in the same row with different capital letters differ significantly (p < 0.05), means in the same column with different small letters differ significantly (p < 0.05).

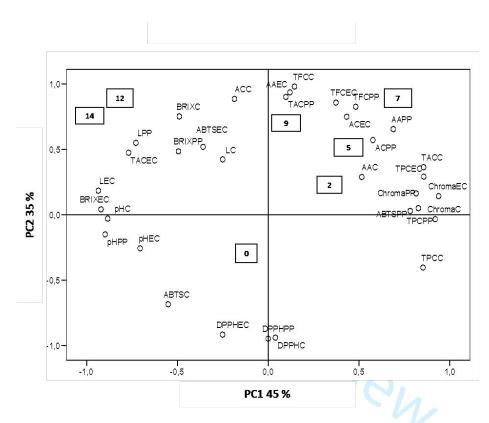


Fig. 1. Loadings and scores plots of principle components analysis (PC1 vs. PC2) on the main sources of variability between samples. PC1 first principle components and PC2 second principle components.

Referee: 1

Q1: The subject of the manuscript entitled "Evaluation of Aloe arborescens gel as new coating for maintain the organoleptic and functional properties of strawberry (Fragaria x ananassa cv. Cadonga) fruits" is innovative. The article is well written and describes the changes on fruit coated with Aloe arborescens gel stored at 3°C/14 days. The titratable acidity, pH, color, weight, soluble solid content, ascorbic acid, total phenols, total flavonoids, total anthocyanins, antioxidant activity by DPPH and ABTS test and texture were evaluated during storage. The materials and methods section is well described, but traditional spectrophotometric methods were used and only 2 methods "in vitro" were applied to determine the antioxidant activity. The results are discussed and the conclusions are supported by these results. To improve the article, I will suggest a more deeply discussion of the results, including more details about the characteristics of A. arborescens that could explain the behaviour of the coating, that is different of the A. vera. Also, it could be addressed a statistical analysis by principal components to set the relationship between the parameters analysed. Actually, the Pearson coefficient could not be the most appropiated statistical tool.

A1: Thank you so much for the comment. As suggested, principal component analysis (PCA) has been carried out, and there is a more detailed discussion of results. Following Refviewer's suggestion we have decided to remove Pearson's correlation coefficient calculation.

Q2: Other changes suggested are:

Line 58: remove the word "antioxidant".

Line 208: add "data not shown"

Line 224: add "Table 1"

Line 319: replace "founded" by "found"

There are some results without significant letters in Table 1.

A2: The statistical analysis was completed in the table 1. Moreover, we have removed the word "antioxidant" from line 58; We have add: "data not shown" In line 208; We have inserted Table 1 in line 224; We have replace "founded" by "found" in line 319;

Referee: 2

Q1: Introduction. The authors develop an investigation based on the use of an Aloe gel to extend the useful life in strawberry, but do not consider more up-to-date information about it, specifically in relation to formulations and possible components in the formulation, plasticizers or others that improve the wetting angle and adhesion of the film, for example. In this case, it would be of interest to include updated information about the use of Aloe gels in formulations for coating in fruits. Then the minimally processed products, their loss of moisture and volatiles are pointed out, but these aspects are disconnected with the argumentation of the Introduction, even more so when the product to be treated is considered in its entirety. There are different format errors, statements not supported by bibliography Line 36 and line 38: without references ... a very large paragraph mentions only one Source, which is not correct.

A1: Thank you so much for the comment. Many works in the literature on the use of edible coatings suggest the use of additives such as antioxidants... to improve the product's shelf-life.

In our experiment we decided to use 100% *aloe arborescens* to make an edible gel, since almost all the literature uses *aloe vera* (also on strawberries). Thus, in order to evaluate fully the effects of the *aloe arborescens* gel, we chose not to use additives. The part not regarding the scientific aims has been eliminated. The references have been added.

Q2: Phytopathological and physiological breakdown problems are also outlined as the most important problems in this type of fruit, the reader thinks that this was the focus of the article, but it is not. Then there is a lack of bibliographical reference in Line 44 that supports his affirmation. A2: The sentence has been improved and the following references have been added: To lengthen the shelf-life of fresh fruit and vegetables a correct choice of packaging must all be taken into account (Sicari et al., 2017; Rizzo et al., 2018; Giuffrè et al., 2019).

Q3: In Line 47: requires bibliographic references that support your claim. A3: Thank you so much for the comment. The references have been added

Q4: Line 53 contains only one reference for all parraf? Missing point in: "line 53 reference to plastics (Andrade Pizarro et al., 2016). These coatings provide a barrier to moisture ",

A4: Thank you for suggestion we have inserted the wrong reference the correct one is Akhtara, J., Omreb, P. K., Ahmad Azad, Z.R.A. (2015). Edible coating for preservation of perishable foods: a review. J. Ready Eat Food. 2, 81-88. This reference is now inserted.

Q5: The authors must define a format criteria for the definition of some terms related to the postharvest of the fruit, such as shelf-life or shelf life (Lines 54, 56, 276).

A5: The terms shelf life was replaced with "shelf-life" in all text.

Q6: Write again sentence in line 56: "Furthermore, edible coatings can contain additives, especially antimicrobial, to help extend the shelf life of the production, as has been widely reported in the literature (Krasaekoopt & Mabumrung, 2008), antioxidants (Rojas-Graü et al., 2008;).

A6: the sentence was re-written.

Q7: To write again, the sentence in line 61 is not understood: "From Aloe two major liquid could be obtained: the yellow latex and the colorless gel, which is obtained from parenchymal cells (Chandegara & Varshney, 2013) which is used in several commercial drinks "

A7: We have removed this sentence and the correspondent reference.

Q8: In Material and Methods section: There is no important information to determine the scope of the results obtained, no mention is made of the concentration used in the applied extract, no prior experiments developed to the respect. Neither do they indicate the performance of the extraction process or greater details of the pasteurization process. Greater background and details of this stage of extraction and preparation of the gel are required. In Page 7, line 108: The authors must explain which is the fundament of selection of treatment with EC treatment or if they count with preliminary results that supports this decision.

A8: We thank for this comment. Necessary information was added to materials and methods section.

Q9: The authors not considered evaluation of effect of modification of the internal atmosphere and gaseous exchange between fruit and external atmosphere like Valverde et al., (2015). Is suggested to evaluate this parameter. It is necessary to detail the technical characteristics of gas permeability of the material used (polypropylene PP boxes used). The authors mention using polypropylene-based anti-fog film (PP-Cartonpack, Rutigliano, BA, Italy), should incorporate more information regarding gas permeability.

A9: We thank for this comment. Unfortunately, in the experiment carried out, we did not measure the internal atmosphere of the PP packages, since our primary objective was to evaluate the effect of the edible coating on the fruit. We are aware that it would have been interesting to add this measurement tour work, and we will certainly bear this suggestion in mind in future studies.

Q10: The authors said in line 110 that "The three different batches of containers (C, PP, EC) were used" ... the sentence is not clear respect to the number of replicates for each treatment, 3 replicates for each treatment or they use one container for treatment?

A10: Three replicates for each treatment were used.

Q11: Details of the ripening stage of the fruits used in the experiment were not provided, nor do they give information regarding the physiological state of the fruit used, degrees brix, acidity, which greatly influences the possibilities of conservation of the product in a refrigerated environment. 250 grams shows approximately 18 fruits in each plastic container. 42 plastic containers, 14 samples without treatment, 14 in anti-Fog PP, 14 treated with Aloe.

A11: Many thanks for your careful observation. We have added the information in the text as presented below: Strawberry fruit (*Fragaria x ananassa*) grown at Reggio Calabria (Italy) were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. After picking fruits were immediately refrigerated at 4-5 °C and transferred to the laboratory in one hour and analyses were conducted. The °Brix was determined in the orchard after picking.

Q12: It is not indicated the age of plants from where they collected Aloe leaves, the physiological age influences the richness of the different components that the authors indicate that they generate protective effects in the coated fruit.

A12: Many thanks for your careful observation. We have added the information in the text as presented below: *Aloe arborescens* leaves were harvested from plants about 6-year old.

Q13: The gel was applied alone, directly to the fruit?. They did not consider any elements?. It is necessary to argue strongly this point. There is abundant scientific evidence regarding the use of Aloe with plasticizers and emulsifiers in a formulation applied by immersion and subsequent drying.

A13: Many thanks for your careful observation. Many works in the literature on the use of edible coatings suggest the use of additives such as antioxidants... to improve the product's shelf-life. In our experiment we decided to use 100% *aloe arborescens* to make an edible gel, since almost all the literature uses *aloe vera* (also on strawberries). Thus, in order to evaluate fully the effects of the *aloe arborescens* gel, we chose not to use additives.

Q14: It is necessary to incorporate information regarding procedures on firmness determination, one or two faces of the fruit?

A14: We thank fort this comment. Necessary information was added to materials and methods

section as presented below:

Fruit firmness was measured as total firmness by a compression test. Each sample was subjected to a two-cycle compression with 5 s between cycles. The highest value of force required to compress the sample during the first compression cycle was recorded as fruit firmness of strawberry. This test measured individual fruit firmness based on the resistance of the flesh to deformation by the probe. Firmness was measured at the equatorial part and on two faces of each strawberry fruit.

Q15: It is necessary to incorporate information regarding the experimental strategy and statistical design considered, design completely random, factorial, etc., these aspects are not mentioned in the manuscript that only shows partially this information related to the treatment of the results. The research does not have statistical design and conclusion in this aspect.

A15: Many thanks for your careful observation.

The main aim of the present work was that of evaluating the effect of the *aloe arborescens* coating on the shelf life of freshly-picked strawberries which were immediately taken to the laboratory. For this reason we did not take into account the experimental strategy or statistical design. We based our choice on a selection of commercially ripe fruit which showed a uniform red surface. As written in Materials and Methods, the strawberries were randomly hand-picked.

Q16: It would be very valuable that the article deepens regarding the possible mechanisms of the alteration of the sugar content in function of hydrolysis of polysaccharides in function of the treatment applied.

A16: Many thanks for your careful observation. Unfortunately, we did not consider this aspect of particular importance. We will certainly bear this suggestion in mind in future studies.

Q17: Line 277: falta referencia bibliografica que respalde la afirmación.

A17: Line 277 now line 286 reported our results. No reference is necessary.

Q18: Line 284: "Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids, 2000)". Falta signo coma antes del año de la referencia.

A18: Comma was inserted before year.

Q19: Line 293. Señalan perdida de componentes en tratamiento control, por alza en respiration pero ellos no lo midieron. Esto debilita el peso de las posibles conclusiones a desarrollar.

A19: Thank you for comments. Taking into account that respiration rate was not evaluated the comment reported in line 293 was removed as well as Reference Ali et al. (2016).

Q20: Line 201: "color. The use of edible coatings such as *A. arborescens* gel make a layer on strawberries skin.... Missing letter in scientific number.

A20: We have checked and corrected.

Q21: In References section: Page15, line 53, the references must have one unique format (see Line 361 and 365).

A21: We have checked and corrected journal abbreviation as follow: "LWT - Food Sci. Technol."

Q22: In section Figures: Figures1 is very small. It does not illustrate the process of extraction or coating on the fruit. It could be dispensed with in the current state, since it does not provide relevant information for the manuscript.

A22: Following the Referee suggesting we have removed Figure 1.

Referee: 3

Q1: Differences in selected properties of 3 groups of strawberries were determined on 6 different days during a period of 14 days storage at 3 °C and 90%? relative humidity, in order to assess the efficiency of a coating with a gel of Aloe arborescens. However, discussion did not consider the actual variation of the selected properties, which are only shown in 2 tables and 1 figure.

A1: We have improved discussion section.

Q2. English language editing is necessary. There are also a lot of print errors.

A2: Native English speaker checked manuscript for edititing. Moreover manuscript was checked for typos.

Q3: 19: "... stored in refrigerator at 3 °C and 50 % relative humidity..." and in lines 110-111: "The three different batches of containers (C, PP, EC) were all refrigerated at 3 ± 1 °C (90 % RH)." Consider revising.

A3: The required corrections were carried out.

described, were refrigerated at 3 ± 1 °C (90 % RH).

Q4: lines 94-95: "The gel obtained from the leaves was filtered to remove fibres and pasteurised at 70 °C for 45 minutes." What kind of filter was used? Was it filtration or sieving?

A4: We thank the referee for this comment. Necessary information was added to materials and methods section as presented below:

The obtained matrix was filtered, to remove fibrous fraction, under vacuum filtration with Buchner funnel using laboratory filter paper. The gel was pasteurized in a thermostatic bath at 70 °C (core) for 45 minutes (Arowora *et al.*, 2013), and allowed to cool immediately to an ambient temperature before further use. The treatment was monitored using a Data Logger (Escort Junior, Astori tecnica, Italy).

Q5: How was pasteurisation performed, in a water bath? Please be precise. 109-110: "... immersing fruits for 1 min in Aloe arborescens gel. After dipping fruits were allowed to air dry for 60 minutes using a high-speed fan." How was the fruit displayed for drying at what temperature? Was there any surface contact of the fruits with other material (wood, plastic)? A5: We thank fort this comment. Necessary information was added to materials and methods section as presented below: The treatment with EC was done by immersing fruits for 1 min at room temperature in *Aloe arborescens* gel. Based on our previous studies, longer dipping times showed no change in results (data not shown). After dipping the fruits were places in stainless steel trays and to airdried for 60 minutes at room temperature for 15 °C. All forty-two samples, subdivide as

Q6: line 137: "Determination of pH, total soluble solid and ascorbic acid content". What about titrable acidity? Was it the pH of crushed fruits or a dilution?

A6: Many thanks for your careful observation. The missed part regards to titrable acidity was added properly to the materials and methods section as showed below:

Determination of pH, titratable acidity, total soluble solid and ascorbic acid content

Strawberries (c.a. 5 g) were ground in a commercial blender, added with 50 mL of H₂O and homogenized using Ultraturrax T-25 (Ika Labortechnik, Janche & Kunkel, Milan, Italy). The mix was

centrifuged at 5000 rpm for 10 min and the supernatant was collected and use for all determination.

Q7: lines168-169: "... TAC content ..." Redundancy.

A7:The method was reported and the redundancy was eliminated.

Q8: 318-319: "Pearson's correlation coefficient evidenced that TPC and Ascorbic acid values are positively linked to the founded antioxidant activity." No correlation results have been reported in manuscript.

A8: Following Referee suggestion we have decided to remove Pearson's correlation coefficient calculation.

We hope the revised manuscript will better suit *the International Journal of Food Science and Technology* but are happy to consider further revisions, and we thank you for your continued interest in our research.

Sincerely, Monica Rosa Loizzo

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