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Effect of plasma activated water and sodium caseinate based coating on the quality of minimally processed cherry tomatoes during storage

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The objective of the work was to preserve the quality of minimally processed (MP) cherry tomatoes during storage combining plasma activated water (PAW) washing treatment and sodium caseinate (SC) based edible coating by:1) optimizing the sodium caseinate coating formulation to reduce the respiration rate of the product; 2) assessing the impact of the combination of (PAW) and coating on the quality of the product during shelf life. Cherry tomatoes (Solanum lycopersicum var. cerasiforme) were coated with SC blended with chitosan (CH) (2% CH/ 4% SC) or guar gum (GG) (8% SC/0,2 % GG; 8% SC/0,3 % GG; 8% SC/0,4 % GG)). Respiration rate results showed that SC/CH coating did not affect the respiration rate of MP cherry tomatoes, whereas SC/GG blends at 0.2 % was able to reduce the respiration rate of almost the 50% at 20°C. However, to avoid the surface stickiness, beeswax (BW) has been included to the matrix. PAW and coating preserved the colour of the product and increased the total polyphenol as well as the microbiological quality of the product during storage.

* 1. Introduction

In the last years, minimally processed fruit and vegetables have gained attention due to the health benefits and convenience. Tomatoe (Solanum lycopersicum L.) is one of the major ingredient in many countries, and its consumption is correlated to low incidence of cardiac disease, due to the presence of lycopene and b-carotene (Ali, Maqbool, Alderson, & Zahid, 2013). However, the shelf life is very short due to its high moisture content, postharvest diseases, increased ripening and senescence (Ruelas-Chacon et al., 2017). It has been estimated that in some developing countries, losses and waste for tomato reached 50% (Yahia, 2009). The respiration rate is one of the most critical aspects because affects the fruit ripening, deterioration and in turn the postharvest shelf life (Ali et al., 2013). The reduction of storage temperature can reduce the respiration rate, but can also be responsible for chilling injury and damage the fruit (Zapata et al., 2008). Another way to control these factors could be the application of edible coating. Edible coatings are a promising sustainable preservation technology for shelf-life extension of minimally processed foods. They can act as semipermeable barriers, modifying the atmosphere surrounding the fruit, and thus controlling the gas exchange, reducing water loss, and maintaining tissue firmness (Gonz et al., 2009). Among the biopolymers, milk proteins, such as casein, are widely used due to their biodegradable nature, availability, low cost, excellent gas barrier, good mechanical properties respect to other biopolymeric packaging material and carrier of bioactive compounds (Khan et al., 2021; Rehan, Volpe, Salucci, Bilal, & Torrieri, 2022; Valentino, Volpe). Casein has been blended with other biopolymers, such as chitosan (Volpe et al., 2017), beeswax and guar gum (Miele, Volpe, Torrieri, & Cavella, 2022) and applied on minimally processed fruit to prolong the shelf life (Volpe et al., 2018). Another important critical aspect that affect the is the deterioration of the tomato, which may also be caused by microbial activity that usually increases during fruit storage (Islam, Lee, Mele, & Choi, 2019). Among the mild technologies, plasma activated water (PAW) represents a good and eco-friendly alternative to washing fruits and vegetables, respect to the traditional chlorinated solutions (Zhao, Patange, Sun, & Tiwari, 2020), since any levels of residual chemicals are kept (Laurita et al., 2021). The objective of the work was to preserve the quality of minimally processed (MP) cherry tomatoes during storage by using a combination of a washing sanitization treatment with PAW and preservation with sodium caseinate based edible coating. In particular, the study was divided in two parts. In the first part, the aim was to optimize the sodium caseinate coating formulation to reduce the respiration rate of the MP cherry tomato. The second part aimed to assess the impact of the combination of PAW and the optimized sodium caseinate edible coating on the quality of the product during shelf life.

* 1. Materials and methods
		1. Materials

Cherry tomatoes (Solanum lycopersicum var. cerasiforme) were bought at a local supermarket and brought to the laboratory within 1 h of the experiment. Sodium caseinate (SC), chitosan (Medium molecular weight deacetylation degree, 75–85%) (CH), glycerol (Gly), guar gum (GG), tween 80 (T80) and span 80 (S80) were purchased from Sigma-Aldrich & Co. (Milan, Italy). Beeswax (BW) was purchased from Agraria Ughetto Apicoltura (Giaveno, Torino, Italy).

* + 1. Coating preparation

SC solutions with protein concentrations of 4% (w/v) and CH solution with a concentration of 2% (w/v) were prepared and blended in proportion of 1:1 as reported by (Volpe, Cavella, Masi, et al., 2017) to obtain SC/CH film forming solution (FFS). Gly was added to the blend to achieve a Gly/solid content ratio of 0.1. To prepare a SC/GG blend, previously 8% of SC (w/v) were dissolved in distilled water for 1,5 h under stirring at 70±2°C; then GG was added in concentration of 0.2% (SC/0.2GG) , 0.3% (SC/0.3GG) and 0.4% (SC/0.4GG) until obtained an homogeneous mixture. Then the blends were cooled down at room temperature. Gly was added in a concentration of 10% (w/w) of the total solid content. SC blended with GG and BW (SC/GG/BW) has been prepared following the procedure of (Miele et al., 2022). Briefly, the coating was composed of 8% of SC (w/v), 0. 2% (w/v) of, 2 % of BW (w/v), 0.25 % (v/v) of T80 and 0.25 % (v/v) of S80.

* + 1. Sample preparation and storage conditions

To study the respiration rate as function of coating composition, cherry tomatoes were previously washed under tap water and dried. Then were dipped by hand into the SC/CH or SC/0.2GG, SC/0.3GG, SC/0.4GG blends for 2 min and then quickly withdrawn and drained. Coated and uncoated tomatoes were dried at 30 °C and 50 % relative humidity (RH) for one hour in circulating air system chamber (MMM Medcenter Einrichtungen GmbH, Munich, Germany) before testing or packaging. To study the effect of PAW and coating on quality of product during storage, cherry tomatoes were washed with PAW for 2 minutes at 30 °C and once dried, were coated with the optimized coating. After the drying process, samples (200 g) were put on a cardboard tray and Naturflex film (Innovia Films, Cumbria, UK) was used to pack the tray. Commercial cherry tomatoes (only packed without washing) were chosen as control sample. The packaged cherry tomatoes were stored at 4°C up to 14 days.

* + 1. Respiration rate measurement

Respiration rate expressed as O2 consumption (RO2) has been measured as reported in (Torrieri, Perone, Cavella, & Masi, 2010) on cherry tomatoes (0.5 kg) at 5, 10, 15 20 and 25 °C. Then, RO2 was measured at 20 °C on coated cherry tomatoes.

* + 1. Headspace Gas Analysis

O2 and CO2 concentration (% v/v) in the package head space were monitored by means of a portable PBI Dansensor A/S (Check Mate 9900 O2/CO2; Ringsted, Denmark) analyzer (accuracy ±0.1%), by sampling with a needle 2–3 mL of gas from the package headspace.

* + 1. Chemico-physical properties

Weight loss was determined by using a gravimetric method. MP cherry tomatoes were weighted before the packaging and at different storage time by using a balance (Mark Ben 3000, Monza, Italia) with an accuracy of 0.01 g. The color of the samples were determined as reported by (Di Giuseppe et al., 2019).

pH, and total soluble solids (TSS) were measured by using tomato juice. To measure the firmness of the samples, a texture analyzer (TMS-Pro Texture Analyzer Food Tech Corporation) has been used by performing a penetration test (load cell: 50 N; penetration rate: 10 mm min-1). Firmness has been calculated as the maximum force required to compress the sample up to a depth of 35%.

* + 1. Total polyphenol content and antioxidant activity

The total phenol content (TPC) of minimally processed tomatoes was determined according to a Folin-Ciocalteu method as reported by (Di Giuseppe et al., 2019). The antioxidant activity (AA) was determined as reported by Volpe et al., (2018) evaluating the free radical- scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

* + 1. Microbiological analysis

Each sample (5 cherry tomatoes) was dipped in 100 ml of quarter-strength Ringer’s solution (Oxoid) and rubbed to allow the dispersion of the microbial cells from the peel to the liquid. From the mother dilution, aliquots of serial decimal dilutions were poured in plates of Plate count Agar (PCA) for the enumeration of total bacterial count (TBC) at 30°C and at 10°C, in Pseudomonas selective agar (PSA) plates to count Pseudomonas spp. and in of Dichloran Rose-Bengal Cholramphenicol agar (DRBC) plates to count yeasts and moulds. PCA, PSA and DRBC plates were incubated: at 30 °C for 48 h and 10 °C for 72 h, at 20 °C for 72 h and at 28 °C for 3-4 days, respectively. The results were expressed in Log CFU/cm2 considering the average surface of 5 cherry tomatoes.

* + 1. Data analysis

The effect of coating on respiration rate and the effect of storage time and process on quality indices of tomatoes has been analyzed by ANOVA by using SPSS v17.0 for Windows (SPSS, Milan, Italy). Duncan’s test and t-test was carried out to find the source of the significant differences within samples (p<0.05). Results and discussions

* 1. Results
		1. Respiration rate measurements

In figure 1A is reported the RRO2 of cherry tomatoes at 5 °C,10 °C, 15 °C, 20° C e 25 °C in air.

Figure 1: respiration rate expressed as RRO2 of minimally processed cherry tomato as function of temperature (A), at 20 °C in air of uncoated (■) and coated (■) with SC/CH blend (B) and at 20 °C in air of uncoated and coated with SC/GG blend at different GG concentration (0.2 %, 0.3 % and 0.4 %) (C).

In Figure 1B the effect of SC/CH coating on the RRO2 and RRCO2 of minimally cherry tomato is reported. The coating did not affect both RRO2 and RRCO2. These results are not in agreement to (Volpe et al., 2017) who found that SC/CH coating was able to reduce of about 50% the respiration rate of minimally processed apples. Since the SC/CH did not affect the RRO2, SC was blended with GG in different concentrations. The addition of only GG to sodium caseinate, led to a reduction of about 37 % in terms of RRO2 of MP cherry tomato compared to the uncoated sample, as shown in the figure 1C. The concentrations of GG did not affect the reduction of respiration rate. Even if the coating was successfully applied on tomatoes, after dried the coating, the product surface resulted sticky. Thus, was necessary to optimize this formulation; SC was blended with GG and BW as reported by (Miele et al., 2022) and applied on tomato. Authors reported the effectiveness of the optimized coating in reducing the respiration rate of strawberries of 17 % compared to the uncoated one. In our study, the optimized coating led to a reduction in RRO2 of MP cherry tomato of 32 % compared to the control at 20 °C (data not shown). SC/GG/BW well adhered to the product surface, without any stickiness and thus was used to study the effect of PAW and coating on MP cherry tomatoes during storage.

* + 1. Headspace gas analysis

Figure 2 shows the headspace gas analysis of packaged MP cherry tomatoes treated with PAW and SC/GG/BW and control.

Figure 2: Headspace gas composition (O2%, (A); CO2%, (B)) of minimally processed cherry tomatoes stored at 4 ◦C for 14 days for control (●) and treated samples (Δ).

The oxygen concentration decreased over time for both samples. After 14 days of storage, the control samples reached 4 ± 1 %, whereas the treated samples reached a value of 11 ± 1 %. As expected, the carbon dioxide concentration increased up to 14 days of storage. For control sample CO2 reached a value of 7.9 ± 0.4 % whereas for treated samples the value was 6.9 ± 0.4 %. These results are expected considering the reduction in respiration rate due to the presence of the SC/GG/BW coating, as reported in the previous paragraph.

* + 1. Chemico-physical properties

Both control and treated MP cherry tomatoes lost weight during storage, reaching about 3.5% of weight loss (data not shown), highlighting that the coating did not affect this parameter. In table 1 the colorimetric parameters for control and treated samples are reported. L\* decreased during storage, starting from 42 ± 1 to 35 ± 2 and 34 ± 2, for control and treated sample, respectively after 14 days of storage. There is a significant effect of time (p<0.05) after 3 days but no effect of treatment. The parameter a\* and b\* increased for both samples over time, showing a significant effect over time. The combination of PAW and coating played an important role in slowing down the increase of a\* and b\* over time, as showed from the significant effect of the combination since the 3rd days of storage. Clearly, this effect can be highlighted by the parameter ∆E, that increased during time reaching a value of 20 ± 2 for control samples and 13 ± 3 for treated samples. Combining mucilage from dragon fruits coating and UV-C irradiation showed similar protective effect on colour change of tomatoes (Razali, et al., 2021). The pH increased during storage time from an initial value of 3.85 ± 0.02 and reaching the highest value (4.56 ± 0.01 and 4.63 ± 0.02 for control and treated, respectively) after 6 days and then decreased again to remain constant until 14 days of storage, without any differences from control to treated sample. The TSS remained constant for the control sample among all the storage time. The treated sample showed a significant (p<0.05) decrease after 3 days but was constant up to 14 days of storage. The firmness significantly decreased over time for both samples without any differences between control and treated sample. This result can be justified by the storage time which is not enough to see difference between samples. Further studied are required to evaluate the impact of the technologies for longer storage time.

Table 1. Color (L\*, a\*, b\*, ∆E), pH, TSS, firmness of control and trated samples (PAW+coating), AA and TPC at different storage time.

|  |  |  |
| --- | --- | --- |
| Sample | Parameters | Time (days) |
| Control |  | 0 | 3 | 6 | 9 | 14 |
|  | L\* | 42±1c | 36±2ab | 37±1b | 35.5±0.8ab | 35±2a |
|  | a\* | 16±3a | 25±2bc | 24±2b | 26±2bc | 27±3c |
|  | b\* | 16±2a | 24±3b | 23±2b | 26±3b | 30±3c |
|  | DE |  | 14±1b | 12±3a | 16±3b | 20±2c |
|  | pH | 3.85±0.02a | 4.48±0.03c | 4.56±0.01c | 4.20±0.02b | 4.24±0.08b |
|  | TSS | 6.1±0.2a | 5.7±0.5a | 5.7±0.4a | 6.1±1.5a | 5.5±0.3a |
|  | Firmness (N) | 24±1b | 24±3b | 22±2ab | 22±3ab | 20±3a |
|  | AA (mg TROLOX eq/g sample) | 0.09±0.01a | 0.12±0.06a | 0.34±0.03c | 0.26±0.08b | 0.17±0.08ab |
|  | TPC (mg GAE/g sample) | 0.97±0.01a | 0.99±0.01a | 1.16±0.01c | 0.96±0.02a | 1.05±0.06b |
| Treated | L\* | 42±1c | 38±2b | 39±2b | 37±2b | 34±2a |
|  | a\* | 16±3a | 19±2a\* | 20±2ab\* | 21±3b\* | 22±2b\* |
|  | b\* | 16±2a | 18±2a\* | 19±3a\* | 20±3a\* | 24±3b\* |
|  | DE |  | 6±2a\* | 7±1ab\* | 9±3b\* | 13±3c\* |
|  | pH | 3.85±0.02a | 4.57±0.02c\* | 4.63±0.05d\* | 4.19±0.03b | 4.24±0.02b |
|  | TSS | 6.1±0.2b | 5.4±0.6a | 5.4±0.5a | 5.5±0.5a | 5.1±0.4a\* |
|  | Firmness (N ) | 23±3b | 23±3b | 22±3b | 19±2a | 19±4a |
|  | AA (mg TROLOX eq/g sample) | 0.09±0.01a | 0.1±0.02a | 0.34±0.03c | 0.46±0.05d\* | 0.2±0.1b |
|  | TPC (mg GAE/g sample) | 0.97±0.01a | 0.99±0.04a | 1.11±0.02b\* | 1.2±0.02c\* | 1.28±0.08d\* |

Different letters show a significant effect of time (p < 0.05); \* significant effect of coating (p < 0.05)

Total polyphenol content and antioxidant activity

In table 1 are reported the TPC and AA of MC tomatoes stored at 4°C over time. TPC for control sample increased, reaching a peak at 6 days and then decreasing again. The treated samples, on the contrary, showed a significant (p<0.05) increase over time up to 14 days. These results are in agreement with Ali et el., (2013) which found a decrease in TPC at 8 days of storage for the control, due to the higher respiration rate, which led to a degradation of certain phenolic compounds. No differences were observed in terms of AA between the control and treated sample.

Microbial count

The results on microbial count are reported in Table 2. Samples showed significant differences on microbial groups analyzed where, in general, treated samples showed the lowest Log CFU/cm2 of microbial groups compared to controls until the end of storage. In details, at 14 days of storage, Y&M, ENT and PSA were completely absent in treated samples while TBC and PB were reduced of about 2 Log in treated samples compared to the controls.

Table 2: Microbial populations (Log CFU/cm2) of cherry tomatoes

|  |  |  |
| --- | --- | --- |
|  Time (days)  | 1Sample  | Log CFU/cm2 on 2microbial group  |
| 0  |   | Y&M  | TBC  | PB  | ENT  | PSA  |
|   | Control  | 1.79±0.04a  | 2.96±0.00a  | 3.06±0.00a  | 0±0.00a  | 0±0.00a  |
|   | Treated | 2.63±0.00b  | 2.93±0.00a  | 2.96±0.00a  | 1.53±0.05b  | 1.48±0.06b  |
| 3  | Control  | 2.18±0.01a  | 2.03±0.01  | 2.99±0.00a  | 0±0.00  | 0±0.00  |
|   | Treated | 1.35±0.11b  | 2.03±0.01  | 2.66±0.00b  | 0±0.00  | 0±0.00  |
| 6  | Control  | 1.18±0.07a  | 3.39±0.00a  | 3.14±0.00a  | 0±0.00  | 0±0.00  |
|   | Treated | 0±0.00b  | 0.40±0.00b  | 0.40±0.00b  | 0±0.00  | 0±0.00  |
| 9  | Control  | 0±0.00a  | 4.27±0.00a  | 3.99±0.00a  | 0.49±0.20  | 0±0.00  |
|   | Treated | 0.86±0.09b  | 3.36±0.00b  | 2.79±0.00b  | 0.10±0.17  | 0±0.00  |
| 14  | Control  | 1.19±0.07a  | 4.31±0.00a  | 4.28±0.00a  | 1.78±0.02a  | 2.03±0.01a  |
|   | Treated | 0±0.00b  | 2.41±0.01b  | 2.73±0.00b  | 0±0.00b  | 0±0.00b |

1: Control, commercial cherry tomatoes; Treated, cherry tomatoes washed with plasma activated water and covered with sodium caseinate based coating. Samples were analyzed at 0, 3, 6, 9 and 14 days of storage at 4°C. 2: TBC, total bacterial count at 30°C; PB, psychotropic bacteria; ENT, *Enterobacteriaceae;* Y&M, yeasts and molds; PSA, *Pseudomonas* spp.

* 1. Conclusion

The combination of PAW and SC/GG/BW edible coating on MP cherry tomatoes stored at 4°C up to 14 days, did not affected the pH, TSS, weight loss and firmness. It was able to well preserve the color changes as well as the microbiological quality during storage and increased the total polyphenol content compared to the control sample. Thus, the combination of PAW and SC based edible coating can be considered a promising alternative mild technology to preserve the safety and the quality of the product during storage.

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