Cluster ions to preserve ready-to-eat table grape during cold storage

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Abstract

The demand for natural fresh-cut products characterized by high quality has promoted the research for non-chemical post-harvest treatments. The aim of this study was to evaluate the effect of air purification with the ion generator Ionny® (Fruit control Equipment, Italy) on fresh-cut table grape quality during post-harvest storage. The air purification was the effect of positively and negatively charged ions generated by the ion generator at atmospheric pressure. The treatment was applied in a storage room on fresh-cut table grape at 0°C for 21 days. Berries were analyzed for weight loss, flesh firmness, skin color, total phenols, acidity, total soluble solids, vitamin C and total yeasts/molds. Results showed that ions treatment positively influenced the quality maintenance of the product, by improving color retention, limiting weight losses and preserving high phenolic content during postharvest storage. Moreover, in cv Red Globe treated berries showed higher soluble solids, acidity, lightness and lower firmness losses, while in cv Italia the ions treatment limited the growth of the microorganisms.

1. Introduction

Ready to eat fruits and vegetable are products with a high convenience value, subjected to minimal processing procedures and, as a consequence, with a very limited shelf-life. Due to the slicing, cutting and peeling, in the fresh-cut products it is observed an increase in respiration rate, ethylene production, enzymatic and microbial activity and therefore a rapid decrease in sensory quality during shelf-life. The increased demand for minimally processed products promoted the research of novel methods of reducing microbial populations and post-harvest losses without sacrificing quality.

Post-harvest life of ready to eat table grape is limited primarily by microbial spoilage, which results, at the end of the storage period, in important economic losses. Harvested bunches are usually stored in the presence of sulfur dioxide. This compound is registered as an adjuvant in most countries, but there is an increasing interest for alternative processing technologies to control post-harvest decay. Nowadays, the studies are focused on meeting the consumer’s demand for safe and high-quality fresh food and consequently, limiting the use of chemical products. For these reasons, the alternative to antimicrobial treatments based on the use of chemical products is physical methods of control. Such methods include the application of hot water treatments (Chiabrando and Giacalone, 2015a; Chiabrando et al., 2018), UV (Guerrero-Beltran et al., 2009), gas content (high CO₂) (Sanchez-Ballesta et al., 2006), hypobaric treatments (Romanazzi et al., 2001), edible coatings (Chiabrando and Giacalone, 2015b), ultrasound (Fava et al., 2011) and electrolyzed water (Chiabrando et al., 2017). A recent and safe technology under study is the use of low-temperature ionized gases (positively and negatively charged ions) at atmospheric pressure (Perni et al., 2008). Air ions are short-lived, lasting only minutes before they lose their charge. Their lifetime is reduced by contacting walls or surfaces, or by interactions with the humidity. Negative ions tend to be smaller with a half-life of several minutes, while positive ones are larger and with a half-life of about 30 mins (Forney et al., 2001). Air ions can be formed artificially through many industrial generators that normally use radioactive sources or electric energy to induce ionization. Air ions also can be generated through coronas by applying a high voltage to a pointed electrode from which ions are expelled (dielectric barrier discharge). High concentration of air ions has been shown to effectively kill or reduce the viability of microorganism like yeasts and molds (Moreau et al., 2008). For these reasons, there is a great potential for the use of ions generators in preventing fruits and vegetable decay by treating the surfaces during post-harvest.
storage period.

This technology offers the advantage of being chemical- and water-free, in addition to being able to operate openly and constantly at atmospheric pressure (Lacombe et al., 2015). Recently, new applications of this technology have been recommended, in order to improve the quality of minimally processed fruit and vegetables.

In fresh-cut apples ions treatment reduced the metabolic activity of the tissue with consequent slowing down of quality decay, in fresh-cut melon peroxidase and pectin methylesterase activities were inhibited and in kiwifruit slices ions treatment improved the pulp color retention during post-harvest storage period (Tappi et al., 2014; Ramazzina et al., 2015; Tappi et al., 2016). Finally, in table grape, a combination of ions and ozone reduced decay losses of about 40% when held at 10°C for 21 days (Forney et al., 2001).

In this context, the aim of this study was to investigate the effects of air ions, generated by a new cluster ion technology, on ready to eat table grape quality and safety.

2. Materials and methods

2.1 Sample preparation, handling and storage

Table grape (Vitis vinifera L.) of “Italia” and “Red Globe” cultivars were obtained from a local market, transported to the DISAFA (Turin University, Italy) laboratory and immediately used. At the time of samples preparation, Italia berries had a soluble solid content of 15.23°Brix, titratable acidity of 2.46 g/L of tartaric acid, skin color (C* and L*) of 8.75 and 36.58 respectively and texture of 0.56 N, measured as an average of 30 berries. Red Globe samples had a soluble solid content of 14.53°Brix, titratable acidity of 1.28 g/L of tartaric acid, skin color (C* and L*) of 5.04 and 32.85 respectively and texture of 0.43 N, measured as an average of 30 berries.

Berries samples were sorted to eliminate undersize or damaged berries, washed to eliminate dirt from the surface and the grape stems were manually removed. For each cultivar and each treatment, 30 boxes (polyethylene box of 13.5 × 17 × 3 cm) of 150 g each were prepared. The boxes were randomly divided in two groups one for the treatment and the other for its control and stored at 0°C during 21 days of storage. The second cabinet with the same characteristics without the ion generator was used as control.

During the storage, three packages for each sample were selected after 7, 14 and 21 days for analytical determinations. Microbiological analyses were performed before the treatment and at the end of the storage period.

2.2 Ions generator inside the storage room

A dielectric barrier discharge (DBD) generator (Ionny®, Fruit control Equipment, Locate di Triulzi, Italy) that produces positive and negative ions together to purify air continuously (400 m³/hour) was used for the treatment. During the trial, a constant concentration of about 600 ions/cm³ was maintained and monitored by an Air Ion Counter (AlphaLab, Inc., Salt Lake City, UT). Ions treatment was run at atmospheric pressure conditions inside the cabinet, ions were widely spread in the room using the axial fan of the Ionny. The ions generator was placed at the top of a refrigerated storage room (cabinet) at a distance of 1.5 m from the berries samples. The discharge was driven between a couple of concentric cylinder-shaped electrodes with air as the source of gas. The electrodes used a high-voltage (2 kV) source, pulsed at 50 Hz from a step-up transformer and operated with an input voltage of 230 V.

2.3 Quality determinations

2.3.1 Weight loss

Weight loss of fresh-cut table grape samples during storage was measured monitoring weight changes of the boxes at 7, 14 and 21 d at 0°C. Weight loss was calculated as the percentage loss of initial weight.

2.3.2 Total soluble solids (TSS) content, pH and titratable acidity (TA)

The TSS content, pH and TA were measured at day 7, 14 and 21 of cold storage for each treatment. TSS (°Brix) was measured using a PRI 1 (ATAGO Co LTD, Tokyo, Japan) digital refractometer in filtered juice extracted from 10 g of fresh berries. TA and pH were determined by adding 50 mL deionized water into 10 ml of filtered juice and analyzing it with 0.1N NaOH up to pH 8.1 with an automatic titrator (Compact 44–00, Crison Instruments SA, Barcelona, Spain). The results were expressed as g/L of tartaric acid. All experiments were performed in triplicate.

2.3.3 Skin color

Skin color was measured with a calibrated Chroma meter (CR-400, Konica Minolta, Inc., Tokyo, Japan) with a CIE C standard illuminant and an observation angle of 2°. The color of ten berries per treatment was determined in CIE L*a*b* color space measuring the
lightness L* (+100 = white, −100 = black), a*(+60 = red, −60 = green), b*(+60 = yellow, −60 = blue) and C* (chroma or saturation) \( C^* = [(a^*)^2 + (b^*)^2]^{1/2} \) parameters (Francis, 1980).

### 2.3.4 Texture analysis

For texture tests, an Universal Testing Machine TAxT2i Texture Analyzer (Stable Micro Systems—SMS, Surrey, UK) equipped with a HDP/90 platform and a 25 kg load cell were used. Skin and pulp hardness was evaluated using a puncture test (Letaief et al., 2008). Samples were placed on the metal plate of the machine with the pedicel placed horizontally in order to be punctured in the lateral face. Hardness means values were calculated from the results of 15 berries for each treatment at day 7, 14 and 21 of cold storage and were expressed in Newtons (N). All the experiments were carried out at room temperature (20°C).

### 2.3.5 Total phenolic content

Total phenolic content was determined with the Folin-Ciocâlteu's reagent method (Kupina et al., 2018) at the start of the experiment and after 21 days of storage. Berries samples (10 g) were homogenized in 25 mL methanol then centrifuged (4°C, 29.430 × g) for 30 mins (Chiabrando and Giacalone, 2015). The supernatants were collected for assay. The extract was oxidized with the Folin-Ciocâlteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 765 nm using a U-5100 Spectrophotometer (Hitachi, Tokyo, Japan) after 30 min at room temperature. The calibration curve was performed with gallic acid, and the results were expressed as g of gallic acid equivalents per 100 grams of fresh berries. All experiments were performed in triplicate.

All standards and reagents were of analytical purity “pro-analysis” and were purchased from SIGMA (Sigma Italiana SRL, Ozzano Emilia, Italy).

### 2.3.6 Vitamin C content

A total of 10 g of fresh berries were homogenized with an Ultra-Turrax T25 (IKA-Werke, Germany) for 2 mins with 10 mL of MeOH/H₂O (5:95 v/v), citric acid (0.1M), acidethylenediaminetetraacetic acid (0.5 g/L) and sodium fluoride (4 mM). The homogenate was filtered and the pH adjusted to 2.2–2.4 adding HCl (4 N). Acidified extract was centrifuged for 5 min at 4°C and the supernatant was filtered through a C18 Sep-Pak cartridge (Waters, Milford, Mass., USA) and a 0.45 μm polytetrafluoroethylene filter (Titan filter 17 mm membrane, SUN-SR). Then, 250 μL of freshly prepared o-phenylenediaminedehydrochloride solution (OPDA, 18.8 mM/L) was added to 750 μL of extract. After 37 min in the dark, the sample was evaluated for ascorbic acid (AA) and dehydroascorbic acid (DHAA) content (González – Molina et al., 2008). HPLC analysis of vitamin C (AA+DHAA) was carried out using an Agilent HPLC 1200 Series (Agilent, Waldbronn, Germany) system consisting of manual injection valve, G1311A quaternary pump, 20-μL sample loop, diode array detector G1315D UV-vis and controlled by Agilent ChemStation software B.03.02. Separations of DHAA and AA were realized with a column Eclipse XDB-C18 (150 x 4.6 mm; 5 μm particle size; Sigma Italiana SRL, Ozzano Emilia, Italy). The mobile phase was MeOH/H₂O (5:95, v/v), 5 mM cetrimide, 50 mM potassium dihydrogen phosphate (pH 4.5). The total run time was 10 mins with a flow-rate of 0.9 mL/min and the detector wavelengths were 348 nm for DFQ detection and 261 nm for AA detection. The vitamin C content was considered as AA and DHAA content and expressed as milligrams per 100 g of fresh sample weight. Reported results values are the mean ± SD of three replicates at day 0 and at the end of the storage period (21 days) for each treatment.

All standards and reagents were of analytical purity “pro-analysis” and were purchased from SIGMA (Sigma Italiana SRL, Ozzano Emilia, Italy).

### 2.4 Yeast and mold evaluation

Total yeasts and molds were evaluated at time 0 and at the end of cold storage. Total yeasts and molds were examined according to the methods described by the Compendium of Methods for the Microbiological Examination of Foods (Vanderzant and Splittstoesser, 1992). A 270 mL of peptone buffered water (Sigma Italiana SRL, Ozzano Emilia, Italy) was added to 30 g of fresh berries sample in a Stomacher® bag using a blender (Stomacher®400 Circulator, Seward, Worthing, UK). Appropriate dilutions were equipped. Rose Bengal agar (Sigma Italiana SRL, Ozzano Emilia, Italy) was used for the yeasts and molds evaluations. All the Petri dishes were incubated at 30°C for 5 days. Results were expressed as decadic logarithm of colony-forming units per gram of fresh weight.

### 2.5 Statistical analysis

All the analyses were carried out at least in triplicate on 3 independent samples and results were reported as mean and standard deviation.

Statistical analysis was performed using one-way Analysis of Variance (ANOVA). Significant differences (p<0.05) between mean values were tested by the Tukey’s HSD test. The data on the microflora were converted into \( \log_{10} \) colony forming units (log CFU/g).
before the analysis. Statistical analyses were carried out using the software STATISTICA for Windows 7 (StatsoftTM, Tulsa, OK).

3. Results

3.1 Weight loss

Minimal processing operations stimulate an increase in berries ripening processes, due to higher fruits respiration rate (Giacalone and Chiabrando, 2013). It is usually considered that fruits and vegetables are deprived of their freshness quality characteristics when they lose more than 3–5% of their weight during the storage period (Robertson, 2006).

As shown in Figure 1, the rate of weight loss of cv Italia increased over the entire storage time in both groups. It can be seen that the total loss in weight did not exceed 4% for all the samples, therefore the berries samples showed good quality in term of freshness even after 21 days of storage. Ions treatment reduced the weight loss amount throughout the entire storage period and recorded the lowest weight loss: about 2.5% compared with 3.7% in the control berries after 21 days of storage. Weight loss was again significantly lower in the Red Globe samples treated with ions compared to the control ones (Figure 2).

Ions treatment reduced significantly weight losses during all the storage time and in both cultivars analyzed; this result confirms the potent inhibition on the respiration/transpiration rate of the ions treatment, suggesting its positive impact on berries quality during postharvest storage (Song et al., 2000; Tappi et al., 2014; Misra et al., 2014).

3.2 Quality parameters

3.2.1 Total soluble solid (TSS) and titratable acidity (TA)

Mean values of the total soluble solid (TSS) and titratable acidity (TA) are shown in Table 1. In both cultivars, slight significant differences emerged between control and ions treated samples means for TSS and TA, but not for every storage time. In terms of TSS, in cv Italia, a higher value was observed in control samples after seven days of storage, while there were no differences within each treatment during the storage period. In cv Red Globe significantly lower values were observed in control samples compared to ions treated during storage (Table 1). In both treatments, there was a significant reduction in sugar content during the storage. Generally, obtained results underlined that the response of grape tissues in terms of chemical modification was quite limited.

Titratable acidity is an essential quality parameter used to evaluate the characteristics of fruits as a result of storage because a significant decrease in TA reflects the senescence of fruits. In this study, the TA decreased significantly in cv Italia in both treatments. Control berries showed higher values of acidity after 7 and 14 days of storage compared to ions treated berries, while at the end of the storage, treated samples recorded the higher values (Table 1). On the contrary, in cv Red Globe, significantly higher TA values were always found in ions treated samples (Table 1). In this case, higher values of acidity suggest a slowing down of the respiration process of the treated samples. The different results in the two considered cultivars, put in evidence a different efficacy of the treatment that was more effective in the reduction of ripening processes on the red berry cv.

However, the influence of the ions treatment on titratable acidity was in accordance with the previous findings in pumpkin puree (Santos et al., 2018).

3.2.2 Skin color

Fruit color is the most important quality factor for
Table 1. Weekly total soluble solids (TSS) and titratable acidity (TA) evaluations of ‘Italia’ and ‘Red Globe’ table grape stored at 0°C and 90% RH under ambient air (control) or ionized air (Ionny).

| Days of Storage | TSS (°Brix) | TA (meq/l) |
|----------------|------------|------------|
|                | Italia     | Italia     |
|                | control    | Ionny      |
| 0              | 15.23±0.21 | 15.23±0.21 |
| 7              | 15.21±0.11 | 15.21±0.11 |
| 14             | 15.33±0.19 | 15.36±0.38 |
| 21             | 15.37±0.12 | 15.13±0.25 |
|                | Red Globe  | Red Globe  |
|                | control    | Ionny      |
| 0              | 14.53±0.32 | 14.53±0.32 |
| 7              | 13.31±0.18 | 13.83±0.15 |
| 14             | 13.83±0.12 | 13.05±0.35 |
| 21             | 13.85±0.26 | 13.85±0.26 |

Data shown are mean ± standard deviation. Means sharing the same letters in rows (A, B, C) and in column for the same cultivar (a, b, c) are not significantly different (Tukey’s HSD test, p<0.05).

Table 2. Weekly color evaluations of ‘Italia’ and ‘Red Globe’ table grape stored at 0°C and 90% RH under ambient air (control) or ionized air (Ionny).

| Days of Storage | C* | L* |
|----------------|----|----|
|                | Italia | Italia |
|                | test   | Ionny  |
| 0              | 8.75±1.57 | 8.75±1.57 |
| 7              | 8.56±1.33 | 6.85±1.15 |
| 14             | 3.86±0.66 | 4.99±0.31 |
| 21             | 4.95±0.52 | 6.53±0.39 |
|                | Red Globe | Red Globe |
|                | test     | Ionny    |
| 0              | 5.04±1.67 | 5.04±1.67 |
| 7              | 5.30±0.99 | 4.47±0.98 |
| 14             | 2.18±0.71 | 2.18±0.71 |
| 21             | 4.91±1.42 | 4.91±1.42 |

Data shown are mean ± standard deviation. Means sharing the same letters in rows (A, B, C) and in column for the same cultivar (a, b, c) are not significantly different (Tukey’s HSD test, p<0.05).

Mean values and standard deviations of the colorimetric parameters C* (color saturation) and L* (skin brightness/lighness) are shown in Table 2. In cv Italia, C* decreased after 14 days of storage and then increased with significantly higher values in the treated berries, which resulted in a fuller and more vivid skin color. The brightness of control berries remained unchanged for all the storage period, instead significantly higher values were shown in the treated samples after 14 and 21 days of storage. Air ions showed a positive effect on color lightness maintenance during storage, that finally resulting in a lighter product with better visual quality.

The same result can be highlighted in cv Red Globe, where higher L* values were detected in treated berries. Also, in this case, ions treatment showed a positive effect on color brightness maintenance during storage. Instead, not the same trend was found for C*. However, similar changes in the external color of fresh-cut cucumber and carrot treated with atmospheric ions have been reported previously (Wang et al., 2012).

3.2.3 Fruit firmness

Fruits texture is a critical quality attribute in consumer’s acceptability of fresh products and ready to eat fruits and vegetable it is one of the most common parameters used to assess the quality. Texture values of control and treated group stored at 0°C for 21 days are shown in Figures 3-4. Throughout the storage, in cv Italia berries firmness remained almost unchanged, while Red Globe control berries showed a more pronounced firmness loss than treated berries.

Generally, the treatment retained the sample firmness in the first storage period for cv Italia and during all the storage time for cv Red Globe, probably because the tissue structure of the berries remained intact. These results indicate that ions treatment may delay berries softening by inhibiting enzymatic and microbial activities and ethylene production (Misra et al., 2014; Tappi et al., 2014).

3.2.4 Vitamin C and total phenolic content

The retention of vitamin C is as an evaluation of the overall nutrient maintenance of fruits because it is highly sensitive to oxidation and starts to reduce immediately after harvest, degrading during storage. The minimally
processed berries samples showed an average of vitamin C content of 9 mg/100 g before the storage. The air ions treatment did not affect vitamin C content, but its significant reduction (about 30% after 21 days of storage) was highlighted without significant difference between the treatments (Table 3).

In both analyzed cultivars, after 21 days of storage, the total phenolic content increased significantly. At the start of the trial, the total phenolic content was about 49 and 56 mg GAE/100 g in Italia and Red Globe respectively. After 21 days of storage phenolic content increased to 103.68 in control berries and to 117.03 mg GAE/100 g in treated Italia berries and to 78.59 in Red Globe control berries and to 82.45 mg GAE/100 g in treated ones. Obtained results are in agreement with recent literature that documented the increase in total phenolic content after ions discharge in apple and pomegranate juices (Table 3) (Herceg et al., 2016; Rodriguez et al., 2017).

3.3 Yeasts and molds evaluation

Microorganisms, such as yeasts and molds, distributed on the fruit surface, are usually the main reason for postharvest deterioration of fruits. The Colony Forming Units (CFU) assay was used to evaluate the postharvest infection of samples after ions treatment during storage (Figure 4). Before the ions treatment, the populations of yeasts/mold on Italia berries were 2.48 and 2.70 log CFU/g, respectively. After 21 days of storage, the microbial count of the control was remarkably (p ≤0.05) higher than that of treated berries, and the reduction of microbial population reached around 1 log CFU/g both for yeasts and molds, demonstrating that ions treatment during storage period could effectively inhibit the microbial contamination on Italia berries and ensure the safety of fruits.

In Red Globe samples before the storage, the total yeasts and molds counts were 2.3 and 2.6 CFU/g respectively. These values showed no significant difference (p ≤0.05) with those found at the end of storage (21 days), indicating that ions treatment, in this case, had no effect on the reduction of microorganisms (Table 4).

4. Conclusion

The effect of air ions, generated by a new cluster ion
technology on ready to eat table grape quality and safety is reported. This study demonstrates that air ions treatment during storage period has the potential to control fruit decay and microbial contamination as well as to maintain fruit quality of postharvest fresh-cut Italia and Red Globe table grape during 21-days storage. To summarize the findings, ions treatment does not adversely affect quality parameters of fruits, on the contrary, treated berries showed a reduction of weight losses and an improve of color retention. Moreover, cv Red Globe treated berries showed higher TSS and TA values, a better lightness of berries and lower firmness losses. At the same time, in cv Italia, the treated air has a positive effect on the containment of yeasts and molds during storage. The maintenance of the quality parameters observed in the present study demonstrated that this treatment can be a new promising alternative to be used as a post-processing technology also with other fresh products.

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