Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.62620

Quality of fresh-cut apples as affected by dip wash treatments with organic acids and acidic electrolyzed water

Alina M. PLESOIANU[1], Violeta NOUR[1,2]*, Felicia TUTULESCU[2], Mira E. IONICA[2]

Abstract

The objective of this study was to investigate the effects of dipping in solutions of citric (2%), benzoic (0.2%), sorbic (0.2%) and ascorbic (0.5%) acids and in acidic electrolyzed water on the quality attributes and surface microbiota of fresh-cut apples, cvs 'Florina' and 'Ionathan', packaged in disposable plastic containers under normal atmospheric conditions during 14 days storage at 8 °C. The colour, firmness, total phenolic content, antioxidant activity and surface microbial load were determined weekly throughout storage. The colour results indicated that acidic electrolyzed water reduced browning while the ascorbic and citric acids were less effective in controlling the enzymatic browning of fresh-cut apples. After 14 days of refrigerated storage, the samples treated with 2% citric acid and acidic electrolyzed water maintained significantly higher firmness, total phenolic content and antioxidant activity than the other treated and control samples. The microbiological analysis revealed that organic acids successfully suppressed bacterial growth throughout the storage period as compared to the control samples. The sorbic and benzoic acids at 0.2% were also effective on yeasts but these dip treatments determined a higher darkening, yellowing and loss of firmness and of antioxidant activity during storage.

Keywords: acidic electrolyzed water; citric acid; benzoic acid; sorbic acid; ascorbic acid; fresh-cut apples.

Practical Application: Extending the shelf-life of fresh-cut fruits by using dip wash treatments with organic acids and acidic electrolyzed water.

1 Introduction

Medical and nutritional research has emphasised the health benefits of fruits and vegetables consumption, including a reduced risk of cardiovascular diseases, certain types of cancer, type II diabetes mellitus and obesity (Slavin & Lloyd, 2012). The fruits and vegetables are relatively low in calories, rich in nutrients and contain high levels of micronutrients, fibres and other bioactive compounds (Vincente et al., 2014). Apples are among the most commonly consumed fruits and they represent an important source of phenolic compounds, organic acids, vitamins, dietary fiber, micro- and macroelements (Souza et al., 2020).

During the last decades, fresh-cut fruits and vegetables have gained great popularity among customers worldwide due to their health benefits and their advantages related to convenience, high nutrition, flavour and freshness (Ramos et al., 2013). The changes of consumers' lifestyle, their interest for new and natural products along with the innovations in processing, product development and new packaging models are the main factors for the growing trend of production and consumption of these types of foods (Oms-Oliu et al., 2010).

Fresh fruits and vegetables are perishable products due to their high water activity, high rate of physiological activity and tender texture (Deng et al., 2019; Nascimento et al., 2020). When these products are minimally processed, they are submitted to unit operations that include selection, cleaning, washing, trimming, peeling, cutting and shredding, sanitizing and packing, which usually damages fruit tissues, increase the susceptibility to microbial attack and shorten the shelf life of fresh-cut fruits and vegetables. Minimally processed fruits and vegetables have only a very short storage life of 4 to 7 days due to ethylene production, respiratory activity, enzymatic and non-enzymatic browning and nutrient release from cells that are stimulated by produce injuries (Ramos et al., 2013). The resulting spoilage of fresh-cut fruits and vegetables during storage is characterized by tissue softening, cut surface browning and other discolorations, decreased nutritional value, loss of texture, translucency, exudation, off-flavour and off-odour development and microbiological growth (Finnegan & O'Beirne, 2015; Ghidelli & Perez-Gago, 2018). Moreover, there has been a dramatic increase in outbreaks of recently reported associated disease with fresh-cut produces caused by the growth of some pathogenic microorganisms on the exposed surface after minimally processing of fruits and vegetables (Callejón et al., 2015). These quality deteriorations and safety concerns have brought an urgent demand for new and improved technologies in order to develop safe fresh-cut fruits and vegetables with high sensory quality and nutritional value.

Dip treatments after peeling and/or cutting into aqueous solutions containing antimicrobial agents, antioxidants, calcium salts or functional ingredients have been investigated to improve quality of fresh-cut fruits and vegetables (Oms-Oliu et al.,

Received 24 Nov., 2020
Accepted 18 Jan., 2021
[1] *Faculty of Food Science and Engineering, "Dunarea de Jos" University of Galati, Galati, Romania*
[2] *Department of Horticulture and Food Science, University of Craiova, Craiova, Romania*
*Corresponding author: vionor@yahoo.com

2010). Organic acids have been widely used in fresh and fresh-cut fruits and vegetables as effective preservatives. The microbial inactivation effects are mainly due to pH reduction, disturbance of membrane transport and/or permeability, anion accumulation, or reduction in internal cellular pH (Ramos et al., 2013). Acidification with citric acid was used to preserve fresh cut apples (DiPersio et al., 2003; Derrickson-Tharrington et al., 2005; Chen et al., 2016) and iceberg lettuce (Akbas & Olmez, 2007) while dipping in solutions of benzoic and sorbic acids was performed to increase the shelf life of broccoli florets (Irkin et al., 2015; Pusik et al., 2018). Citric and ascorbic acids have been used in fresh and fresh-cut fruits and vegetables to control browning (Li-Qin et al., 2009). The antibrowning activity was attributed to the inhibition of the polyphenol oxidase enzyme activity in damaged cells (Zhou et al., 2020).

Acidic electrolyzed water (AEW) is considered as an effective disinfectant in food decontamination and preservation due to its ability to inactivate several pathogenic and spoilage microorganisms with minimal negative effects on human and on the organoleptic and nutritional quality of foods (Gil et al., 2015). Its disinfection efficacy against different foodborne pathogens is due to the low pH (2.5-3.5), high oxidation reduction potential (ORP, >1000 mV), and the presence of HOCl (Hao & Wang, 2019). AEW is reported to provide effective inactivation of microorganisms on fresh-cut apples (Graça et al., 2020), fresh-cut pears (Graça et al., 2017), carrots (Koide et al., 2011) and on fresh ready-to-eat vegetables and sprouts (Issa-Zacharia et al., 2011).

The objective of this study was to investigate the effects of dipping in solutions of organic acids and in acidic electrolyzed water on quality attributes and surface microbiota of fresh-cut 'Florina' and 'Ionathan' apples packaged in disposable plastic containers under normal atmospheric conditions during 14 days storage at 8 °C.

2 Materials and methods

2.1 Reagents

Analytical grade chemicals: methanol, Folin-Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and sodium acetate were purchased from Sigma-Aldrich (Germany); benzoic acid, sorbic acid, ascorbic acid and sodium carbonate from Merck (Darmstadt, Germany).

The acidic electrolyzed water used in this study is acidic water produced from Kangen Water Type LeveLuk SD 501 apparatus purchased from Enagic (Dusseldorf, Germany).

2.2 Plant material

Apples (*Malus domestica* Borkh.) cvs. 'Florina' and 'Ionathan' obtained from a wholesale market were used for this study. These cultivars were chosen for the experiment based on their wide popularity and their rapid browning after cutting. The fruits were stored at 8 °C before processing. They were sorted to eliminate damaged or defective fruit and washed with tap water, then drained and placed on filter paper.

2.3 Fruit preparation and treatment

Apples were manually sliced transversely (20 mm thick), cut in cuboids (2-3 cm) with a sharp knife and all core tissue was removed. The treatments were done by dipping the apple cuboids for 5 minutes into the test solutions. The solutions tested included 2% citric acid (CA, pH=1.67), acidic electrolyzed water (AEW, pH=3.54), 0.2% benzoic acid (BA, pH=2.62), 0.2% sorbic acid (SA, pH=2.78) and 0.5% ascorbic acid (pH=3.50). Samples were then drained, placed in disposable plastic containers (750 mL capacity, eight cuboids/container) and stored at 8 °C for two weeks. Control samples (C) were dipped in distilled water. Two replicates of each treatment were prepared for each experiment, and two experiments were performed. Colour, firmness, titratable acidity, total phenolic content, DPPH antioxidant activity and microbiota were measured at 0, 7, and 14 days of storage.

2.4 Firmness measurement

The flesh apple firmness was measured with a GY-3 fruit penetrometer (Sundoo Instruments, Zhejiang, China) fitted with a round plunge (8 mm diameter). An even force was applied to the penetrometer tip to penetrate the apple tissue. When the probe advanced into the tissue to the required scale, the force was removed, and the force gauge reading was recorded. Four slices from each bag were selected randomly and evaluated for firmness measurement on three sides and the average value was reported in $kg \cdot cm^{-2}$.

2.5 Colour analysis

Cut apple surface colour values (CIE L*, a*, b*) were directly measured with a PCE-CSM1 reflectance colorimeter (PCE Instruments, UK) calibrated against a white standard. The analysis was performed on four samples from each treatment with three readings on each sample. A decrease in L* value indicated a loss of whiteness, and a more positive a* value indicated that browning had occurred, whereas a more positive b* indicated yellowing.

2.6 Total phenolic content

The extraction was done by taking 3 g of fruit tissue homogenized with 10 mL of methanol in an ultrasonic bath for 60 min at room temperature. After filtering, the process was repeated for residue. Finally the extracts were combined and diluted to 50 mL with methanol. Total phenolic content was assessed according to the Folin-Ciocalteu phenol reagent method. 100 μL of each extract were mixed with 5 mL of distilled water and 500 μL of Folin-Ciocalteu reagent. After 2 min, 1.5 mL of 20% sodium carbonate was added and the reaction mixture was diluted with distilled water to a final volume of 10 mL. After incubation for 30 min at 40 °C, the absorbance was measured at 765 nm on a Varian Cary 50 UV spectrophotometer (Varian Co., USA). The results were expressed as mg gallic acid equivalents (GAE) per 100 fresh weight (fw).

2.7 Antioxidant activity

The antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazil) assay. The extraction of samples

was made according to the same protocol described for total phenolic content. 50 µL fruit extract was mixed with 3 mL DPPH methanolic solution (0.004%). After incubation in the dark for 30 min at room temperature, the absorbance was measured at 517 nm on a Varian Cary 50 UV-VIS spectrophotometer. The results were expressed in mmol Trolox per 100 g fresh weight (fw).

2.8 Microbiological analysis

The microbiological analysis was performed by conventional culture techniques. The microorganisms were sampled from the flesh fruits surface using sterile swabs and inoculated on nutrient agar plates. Every inoculated plate was incubated at 30 °C for 48 hours. After the incubation time, the grown colonies were counted, and their morphology was studied. The observations were made on the cultural characteristics of the colonies (appearance time of colonies, degree of development, type of colonies, appearance, colour, degree of transparency, consistency). The analysis was completed with microscopic examination. The microbial load on the fruit surface was calculated from the number of colonies grown on the plates and it was expressed in CFU·cm^{-2}.

2.9 Statistical analysis

The results are reported as mean ± standard deviation. The effect of storage time and treatment were analysed using the least significant difference (LSD) test and statistical significance was identified at $p < 0.05$. The data were analysed with Statgraphics Centurion XVI software (StatPoint Technologies, VA, USA).

3 Results and discussion

The colour, i.e. browning at the cut surface, was found to be a critical quality parameter determining the shelf-life and the purchase decision of the fresh-cut fruits. The influence of chemical treatments on the colour of fresh-cut apples was evaluated by monitoring the changes in colour values (L*, a*, b*) at the surface of apple pieces stored at 8 °C for 14 days. The CIELab parameters are frequently used in monitoring the browning of cut apple surfaces, a decrease in L* value and an increase in a* value being indicative of browning (Soliva-Fortuny et al., 2001; Rojas-Graü et al., 2008).

The L* values of fresh-cut apples decreased during storage for all treatments (Figure 1). This trend was also found in previous studies (Soliva-Fortuny et al., 2001; Raybaudi-Massilia et al., 2007) and it was attributed to enzymatic browning caused by tissue damage with consequent enhanced contact between enzymes and substrates (Rojas-Graü et al., 2008). A lower decrease in L* values of treated samples compared to non-treated samples during storage showed that the chemical treatment preserved the natural colour of the fresh-cut apples.

The highest decline in L* levels was observed for BA treated samples from both varieties throughout the storage period while the lowest was found for AEW treated samples, thus confirming the effectiveness of AEW as antibrowning agent reported in previous studies (Wang et al., 2007; Li et al., 2017). At the end of the storage period L* values were 70.7 and 72.0 for non-treated samples and 75.6 and 73.5 for AEW treated samples for 'Florina' and 'Ionathan' cultivars, respectively.

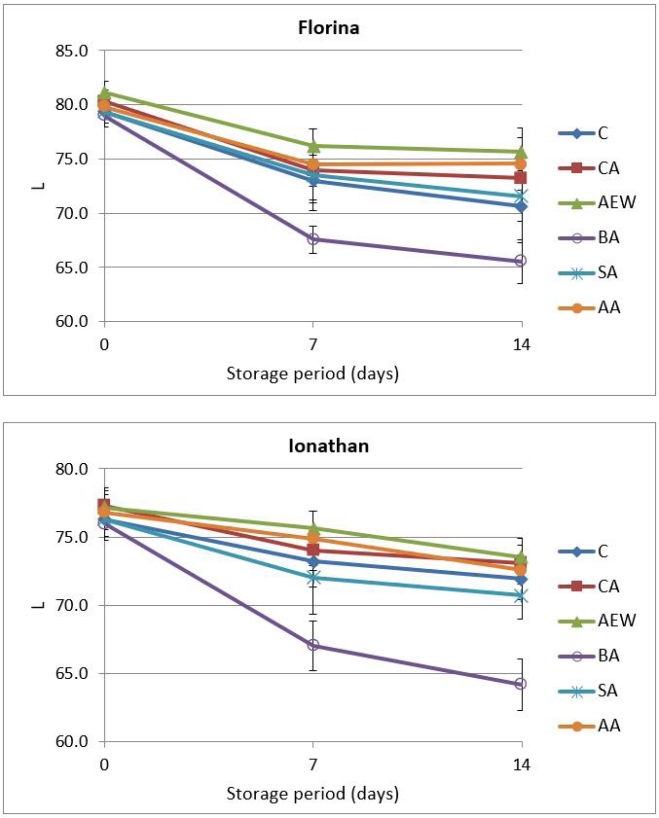
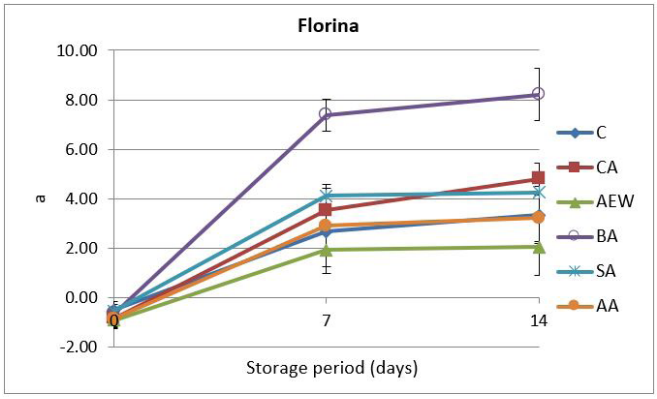


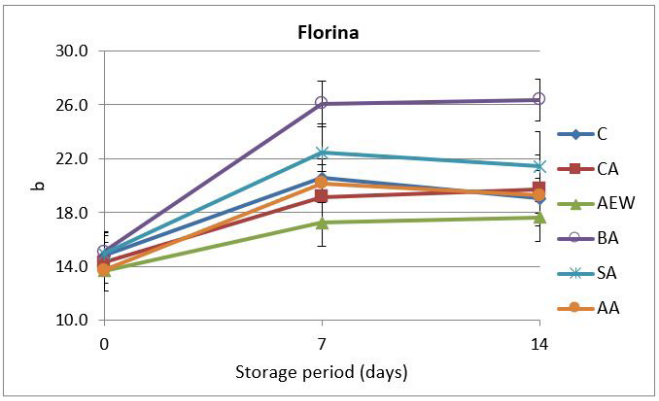
Figure 1. Effect of chemical treatments on L* values of fresh-cut apples ('Florina' and 'Ionathan' cultivars) during 14 days storage. Fresh-cut apple cuboids were dipped for 5 min in tap water (C-control), 2% citric acid (CA), acidic electrolyzed water (AEW), 0.2% benzoic acid (BA), 0.2% sorbic acid (SA) and 0.5% ascorbic acid (AA) and then stored at 8 °C. Data represent means of three replications ± SD.

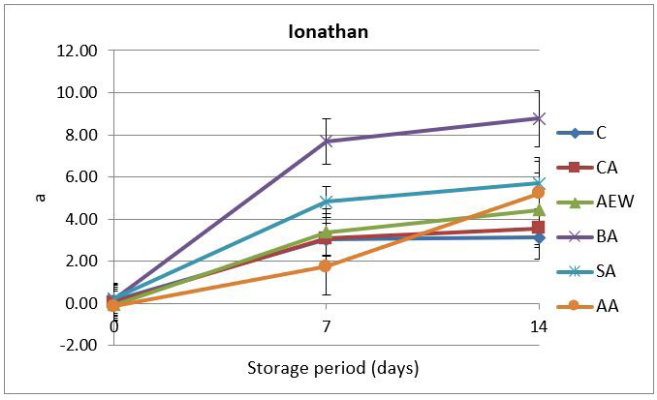
Although several previous studies have reported antibrowning activities of citric and ascorbic acids (Li-Qin et al., 2009), in our study the L* values measured on samples treated with citric or ascorbic acid did not differ significantly ($p < 0.05$) from those for the control samples. Our results are in agreement with the previous studies reporting that ascorbic acid was not completely effective to control enzymatic browning of fresh-cut apples (Rojas-Graü et al., 2008) and pears (Oms-Oliu et al., 2006), while Chen et al. (2016) reported that citric acid alone aggravated browning of fresh cut apples.

The effectiveness of AEW as antibrowning agent also became evident according to the results obtained in the a* values, as can be seen in Figure 2. The a* values increased during the two weeks storage period in all samples. Similar variations have been reported in previous studies on fresh cut apples (Rocha & Morais, 2002; Rojas-Graü et al., 2008). The highest increase of a* values was observed for apple pieces dipped in 0.2% benzoic acid solution, reaching values of 8.2 and 8.7 for 'Florina' and 'Ionathan' cultivars respectively after two weeks of storage and suggesting a fast darkening. Non-significant differences for a* values were found between control samples and those treated with citric and ascorbic acids.

The b* values of apple samples of all treatments increased during storage (Figure 3). The b* values were significantly higher







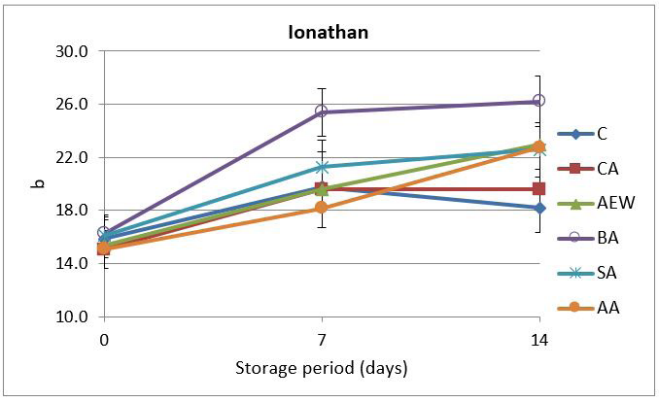


Figure 2. Effect of chemical treatments on a* values of fresh-cut apples ('Florina' and 'Ionathan' cultivars) during 14 days storage. Fresh-cut apple cuboids were dipped for 5 min in tap water (C-control), 2% citric acid (CA), acidic electrolyzed water (AEW), 0.2% benzoic acid (BA), 0.2% sorbic acid (SA) and 0.5% ascorbic acid (AA) and then stored at 8 °C. Data represent means of three replications ± SD.

Figure 3. Effect of chemical treatments on b* values of fresh-cut apples ('Florina' and 'Ionathan' cultivars) during 14 days storage. Fresh-cut apple cuboids were dipped for 5 min in tap water (C-control), 2% citric acid (CA), acidic electrolyzed water (AEW), 0.2% benzoic acid (BA), 0.2% sorbic acid (SA) and 0.5% ascorbic acid (AA) and then stored at 8 °C. Data represent means of three replications ± SD.

in the BA and SA treated samples, showing a yellowing effect of benzoic and sorbic acids on fresh cut apples surface.

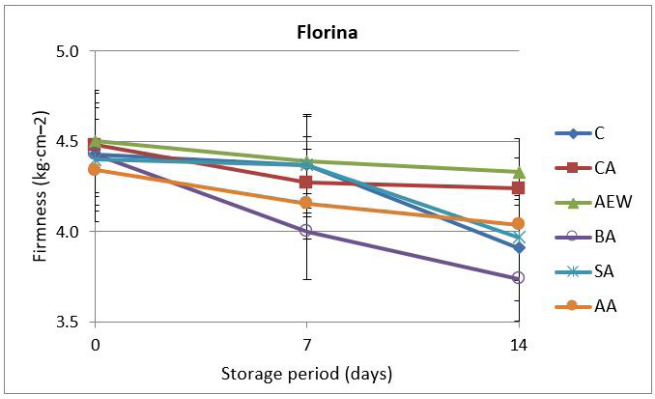
Figure 4 presents the firmness of fresh-cut apples stored for 14 days at 8 °C. As found in previous studies, the firmness of apple slices decreased during storage (Fan et al., 2005) but it was affected by variety, treatment and storage time. The apple variety is a significant factor influencing the change of firmness through storage time (Raybaudi-Massilia et al., 2007). Firmness of 'Florina' samples was significantly higher than that of the 'Ionathan' samples over the 14 days of storage. After 7 days of storage, for both apple varieties, there was no significant difference in the firmness among the treated and non-treated samples while after 14 days of storage, the samples treated with 2% citric acid and acidic electrolyzed water maintained significantly greater firmness than the other treated and control samples. Contrariwise, Chen et al. (2016) reported that 0.5% citric acid had no significant effect on the firmness of fresh-cut apples. The results show that, for both apple varieties, AEW treated fresh-cut apples had the best firmness while BA treated samples had the lowest firmness after 14 days of storage.

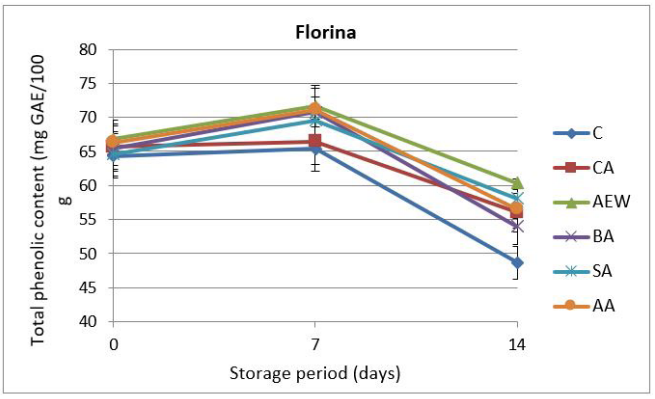
On the processing day, the mean of total phenolic contents were 65.4 and 45.3 mg GAE/100 g for 'Florina' and 'Ionathan' cultivars, respectively (Figure 5). A significant difference ($p < 0.05$) in the total phenolic content was observed between the apple cultivars, as previously reported in other studies (Silva et al., 2019).

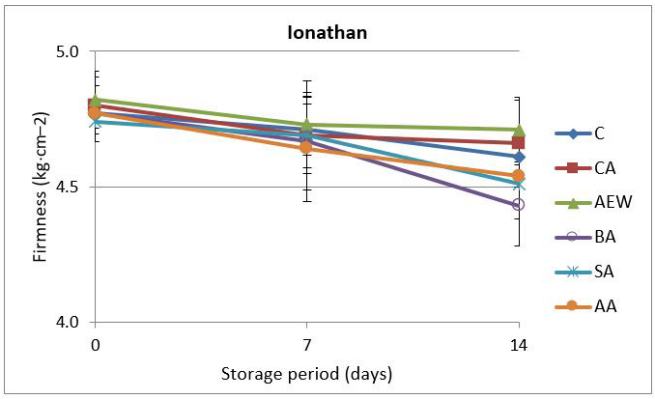
For both apple cultivars, there was a slight increase in the total phenolic content of apple cuboids during the first week of storage, followed by a decrease.

After 7 days storage, no significant differences were found between the chemical treated and non-treated samples while at the end of the storage period total phenolic levels were higher for chemical treated samples compared to control samples. Among treatments, AEW treated samples achieved the highest total phenolic content after two weeks of storage, probably because of the antioxidant activity of AEW that prevented a high phenolic degradation. Chen et al. (2019) reported also that AEW treatment enhanced the storability of harvested blueberries via increasing ROS scavenging capacity.

Figure 6 presents the DPPH radical scavenging activity results of apple cuboids during the cold storage. The antioxidant activity values determined by DPPH on the processing day were 2.67 and 1.53 mmol Trolox/100 g fw for 'Florina' and 'Ionathan' cultivars, respectively. The general trend of the antioxidant activity throughout 14 days at 8 °C was decrease compared to the levels on the processing day. There were no significant ($p < 0.05$) DPPH







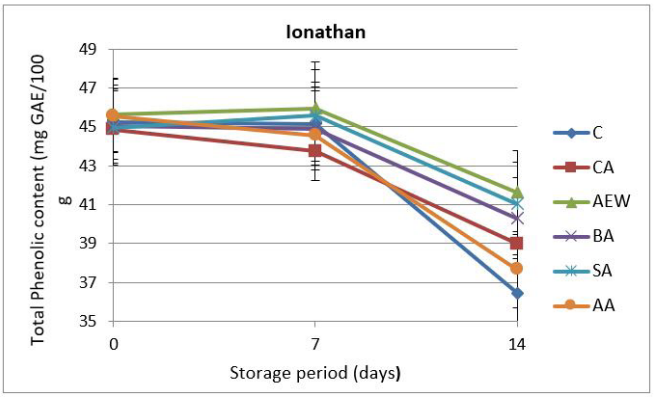


Figure 4. Effect of chemical treatments on firmness of fresh-cut apples ('Florina' and 'Ionathan' cultivars) during 14 days storage. Fresh-cut apple cuboids were dipped for 5 min in tap water (C-control), 2% citric acid (CA), acidic electrolyzed water (AEW), 0.2% benzoic acid (BA), 0.2% sorbic acid (SA) and 0.5% ascorbic acid (AA) and then stored at 8 °C. Data represent means of three replications ± SD.

Figure 5. Effect of chemical treatments on total phenolic content of fresh-cut apples ('Florina' and 'Ionathan' cultivars) during 14 days storage. Fresh-cut apple cuboids were dipped for 5 min in tap water (C-control), 2% citric acid (CA), acidic electrolyzed water (AEW), 0.2% benzoic acid (BA), 0.2% sorbic acid (SA) and 0.5% ascorbic acid (AA) and then stored at 8 °C. Data represent means of three replications ± SD.

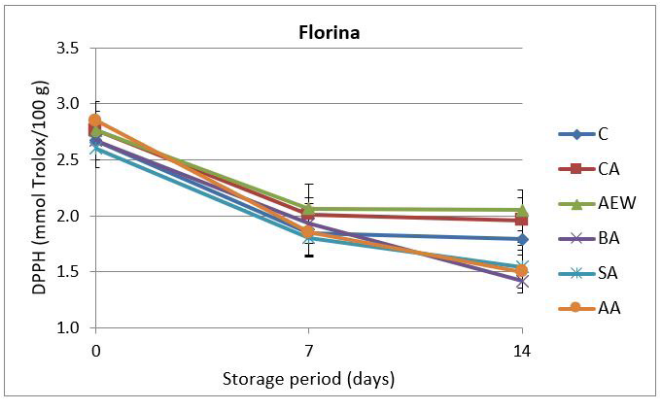
antioxidant activity differences between control samples and all treatments for both apple cultivars after 7 days of storage.

At the end of the 14 days storage period, AEW and CA treated samples showed higher antioxidant activity values while SA, BA and AA treated samples showed lower antioxidant activity values than control samples.

The microbiological analysis performed on the control and treated fresh-cut apples revealed significant differences between treatments. At the beginning of the storage period, the highest microbial load was recorded on the control samples (80 CFU·cm^{-2}) consisting of yeasts and bacteria of the *Bacillus* and *Staphylococcus* genera. After 7 and 14 days of refrigerated storage, isolated as well as confluent colonies were formed on the culture medium from control samples, giving a lawn of colonies on the plate.

Immediately after treatment, the surface microbial load of the samples dipped in 2% citric acid was low (2 CFU·cm^{-2}) compared with the control samples (80 CFU·cm^{-2}) and consisted only of yeasts. During the storage period, increases in microbial count were observed both in control and CA treated samples. The citric acid completely inhibited bacteria growth throughout the storage period as compared to the control samples. The reduction in the bacterial population of fresh-cut apples as a result of the immersion in citric acid solutions has been reported in previous studies and it was partially attributed to its pH-lowering ability (DiPersio et al., 2003).

Dip treatment of fresh-cut apples with acidic electrolyzed water led to a considerable reduction in the number of microorganisms (5 CFU·cm^{-2}) compared to the control samples. However, *Bacillus* and *Staphylococcus* bacteria were found on the surface of AEW treated samples. After 7 days of refrigerated storage, the microbial load increased tenfold on AEW treated samples while the number of *Bacillus* bacteria remained constant. The yeasts load increased during 14 days refrigerated storage on AEW treated samples, the number of colony-forming units exceeded 300 CFU·cm^{-2}, the colonies enlarged and became confluent. However, the AEW treatment resulted in a significant reduction of the *Staphylococcus* and *Bacillus* load as compared with the control samples. The capacity of acidic electrolyzed water to provide effective inactivation of microbials was previously reported in fresh-cut apples (Graça et al., 2020), sliced carrot (Koide et al., 2011), and fresh-cut broccoli (Navarro-Rico et al., 2014). All these studies have highlighted the growing interest in the use of AEW for its high antimicrobial efficacy and minimal effects both on the organoleptic and nutritional quality of food and on human health.



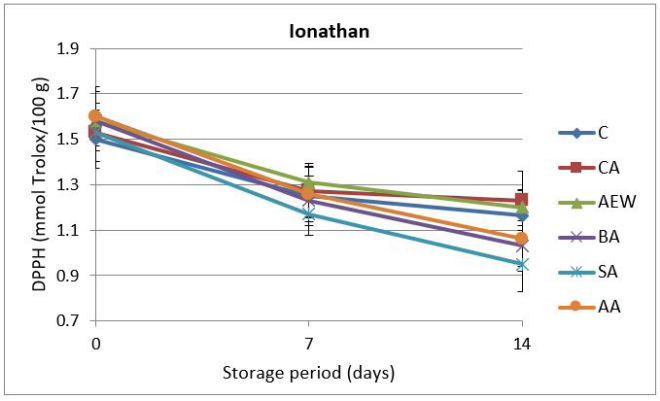


Figure 6. Effect of chemical treatments on DPPH radical scavenging activity of fresh-cut apples ('Florina' and 'Ionathan' cultivars) during 14 days storage. Fresh-cut apple cuboids were dipped for 5 min in tap water (C-control), 2% citric acid (CA), acidic electrolyzed water (AEW), 0.2% benzoic acid (BA), 0.2% sorbic acid (SA) and 0.5% ascorbic acid (AA) and then stored at 8 °C. Data represent means of three replications ± SD.

Benzoic acid successfully suppressed bacterial and yeasts growth on fresh-cut apples throughout the storage period. No bacteria grew on nutrient agar plates from BA treated samples during 14 days of refrigerated storage. Only a small number of yeast colonies have grown from samples dipped in 0.2% benzoic acid (3 CFU·cm^{-2}) and this remained constant even after 14 days of refrigerated storage. A decrease in the size of the colonies was noted at the end of the storage period, indicating an inhibition of yeasts determined by benzoic acid.

The treatment with 0.2% sorbic acid provided an effective inactivation of microorganisms on fresh-cut apples, resulting in a significant lower microbial count than those of the control samples throughout the storage period. At the beginning, a significantly lower number of yeast viable cells (25 CFU·cm^{-2}) were found on SA treated samples as compared with control samples. After 7 and 14 days of storage, no microorganisms grew on the agar culture medium from SA treated samples.

Dipping in 0.5% ascorbic acid resulted also in a considerable reduction of the microbial load as compared to the control samples. However, after treatment, *Bacillus* bacteria (1 CFU·cm^{-2}) and yeasts (16 CFU·cm^{-2}) were found on the fruit surface. Morphological analysis revealed larger yeast colonies as compared to those from the control and the other treated samples. After refrigeration for 7 days, there is a reduction in the number of yeasts as compared to the initial stage and complete inhibition of bacteria while after 14 days, numerous small colonies of yeasts grew, became confluent and difficult to count. At the end of the storage period, the microbial load of the AA treated samples was higher compared to the control samples but yeasts predominated on these samples, whereas on control samples the microbiota was dominated by *Staphylococcus* bacteria. Beside the pH decrease, that may stress bacterial cells and inhibit their survival, the antibacterial activity of ascorbic acid may relate to its ability to react with metal ions and generate hydroxyl radicals, which attack biological molecules (DiPersio et al., 2003).

4 Conclusion

According to the results obtained in this study, dipping in acidic electrolyzed water and 2% citric acid solution enhanced the storability of fresh cut apples by inhibiting browning and microbial growth and by reducing the loss of firmness, of total phenolic content and of antioxidant activity during storage. Acidic electrolyzed water was more effective than citric and ascorbic acids in controlling enzymatic browning of fresh-cut apples. The treatment with 0.5% ascorbic acid inhibited bacteria but promoted yeast growth during storage. Dipping in solutions of 0.2% benzoic and sorbic acids provided an effective inactivation of microbials on fresh-cut apples throughout the storage period. However, these treatments resulted in a higher darkening, yellowing and loss of firmness and of antioxidant activity during storage. Further studies are required to investigate if combinations of these treatments could be effective for the better maintenance of quality during storage of the fresh cut apples.

Acknowledgements

This work benefited from the networking activities within the European funded COST ACTION CA18113 - Understanding and exploiting the impacts of low pH on micro-organisms.

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