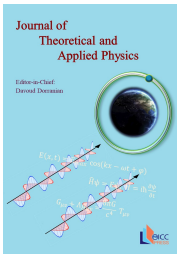

Volume 17, Issue 2, 172319 (1-10)

Journal of Theoretical and Applied Physics (JTAP)

https://dx.doi.org/10.57647/j.jtap.2023.1702.19

Investigation of plasma-activated water effects on preservation and physicochemical properties of Petroselinum crispum and Lepidium sativum

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Received 23 November 2022; Accepted 22 January 2023; Published online 26 January 2023

Abstract:
The present study aims at highlighting plasma, plasma-activated water (PAW) in particular, as a means of alternative, an innovative and unique method for its application in different aspects of food and vegetable processing and preservation. To investigate the effect of PAW on nutrient retention in vegetables, an experimental set was used to produce PAW that processes deionized water (DI). The concentrations of reactive species in PAW were calculated to compare their effect on nutrient retention. Then, the vegetables were washed with DI and PAW for 20 minutes. The samples were stored at 4 °C for 14 days. Immediately after washing and on the seventh and fourteenth days, nutrient content levels were measured. The results showed that using PAW causes the nutrients to remain in the vegetables for a longer time. We show that nutrient persistence in vegetables depends on the concentration and the type of reactive species in PAW.

Keywords: Plasma-activated water (PAW); Preservation; Fresh vegetables

1. Introduction

Consumption of most fruits and vegetables due to the high concentration of nutrients and biologically active compounds has many health and nutritional benefits [1]. One of the problems of the food and drug industry is the spread of microbial strains resistant to drugs and antibiotics. Today, due to the toxic and carcinogenic properties of chemical and synthetic compounds, the use of medicinal plants and fresh vegetables to treat chronic diseases and provide the nutrients needed by the body has attracted the attention of many researchers. Therefore, the use of fresh vegetables is significant because they are a rich source of phenolic and flavonoid compounds and have antioxidant properties [2]. Many study has demonstrated that the source of phenols and flavonoids, and other nutrients needed by the body in different parts of the world depends on the type of diet of the people in the region. One of the most important sources of these nutrients is fresh vegetables. Therefore, using new methods to prevent the growth of microorganisms and reduce the nutrients in vegetables and increase the shelf life of fresh vegetables over time is of great importance [3]. Every step the product goes through, from cultivation to store, is vital in terms of quality and safety. In general, fresh vegetable packaging instructions include a washing or disinfection step to remove dirt, pesticide residues, and microorganisms that cause quality loss, nutrient loss, and rot in fresh vegetables [4]. There are several ways to wash fresh vegetables. Chemical-based methods such as chlorine-based chemicals [5], chlorine dioxide [6], organic acids [7], hydrogen peroxide [8], calcium-based solutions [9], ozone [10], and electrolyzed water [11] are the most common methods. The use of these methods due to concerns about the residue of these chemicals in vegetables and the effectiveness of these methods, in the long run, has caused some European countries to limit their use. Therefore, it was necessary to look for new and effective methods. Several new preservation methods are being expanded to satisfy

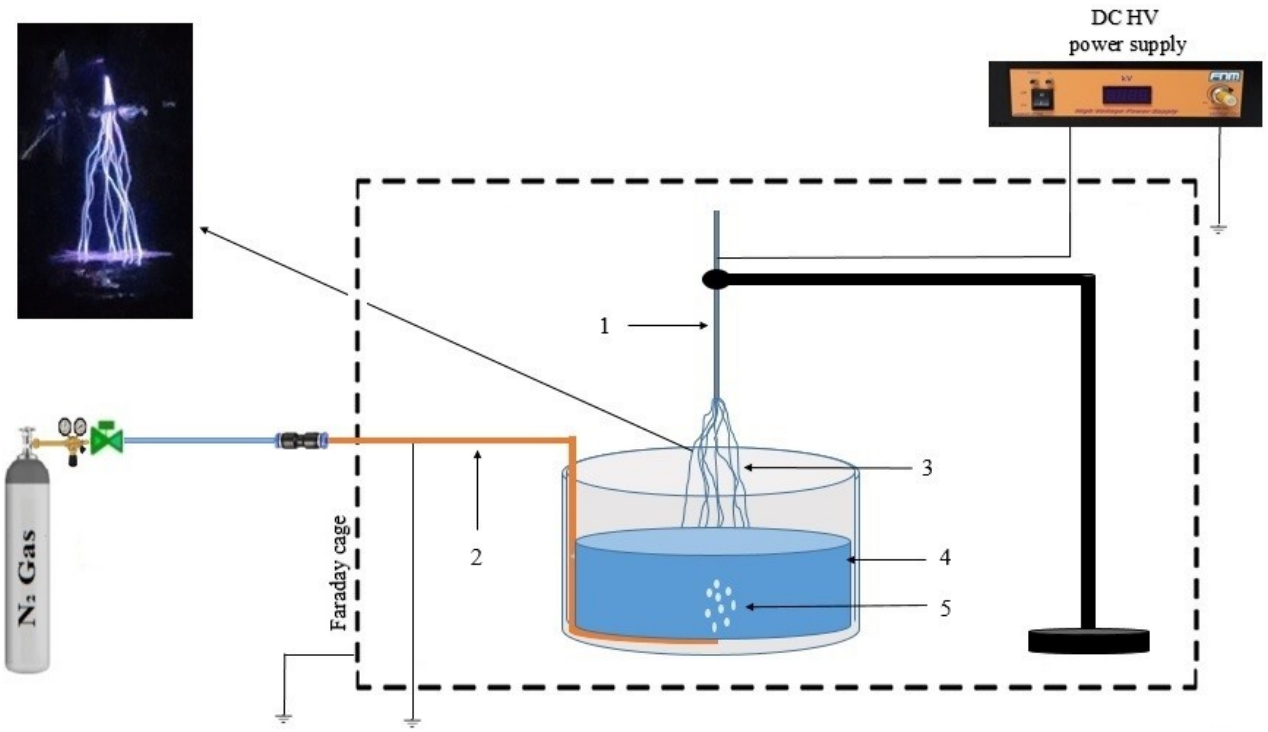

Figure 1. Schematic diagram of the experimental setup. 1: high-voltage electrode, 2: ring electrode, 3: plasma discharge, 4: sample, 5: gas outlet.

current demands of consumer satisfaction in nutritional value, freshness, taste, and sensory aspects, convenience in use, no preservatives, low energy requirement, environmental protection, and economic preservation techniques. Better understanding and utilization of these conventional and complicated preservation techniques could help to extend high-quality, safe products by better control of the production and storage processes and efficient selection of ingredients in the final products [12–16]. With this in mind, and given the characteristics of atmospheric pressure plasma, it can be introduced as an innovative technology for increasing the storage time and nutrient retention of fruits and vegetables.

Atmospheric pressure plasma is a chemically active medium, containing electrons, atoms and excited ions, and free radicals. These reactive species include atomic oxygen, ozone, reactive nitrogen and oxygen radicals (RONS), and species such as hydroxyl and hydrogen peroxide. These reactive species react with microorganisms by destroying the cell wall or damaging DNA, or altering cell metabolism, leading to the inactivation of microorganisms. This phenomenon has been the source of many applications in medical sciences and the food industry. So, in recent years, many research have investigated the interaction of plasma with

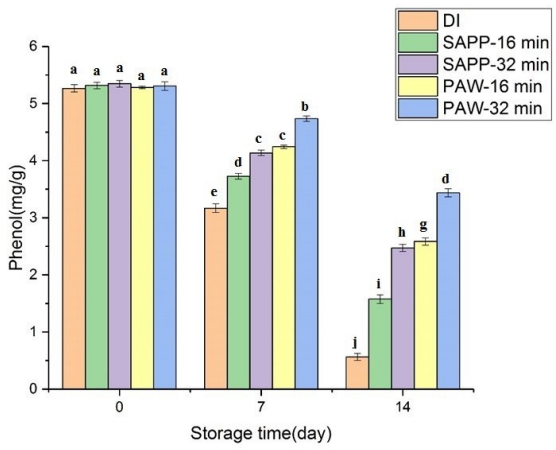

Figure 2. Changes in phenolic content of P. crispum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

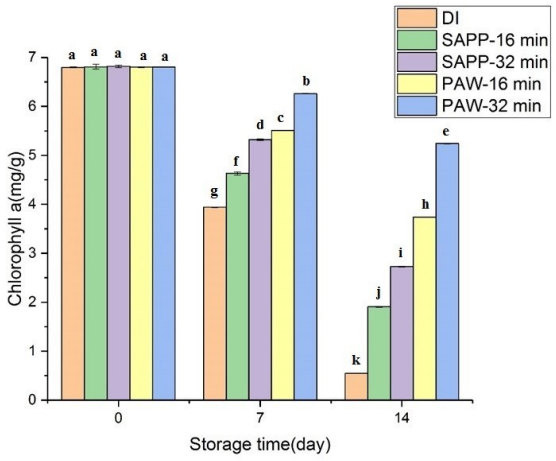

Figure 3. Changes in phenol content of L. sativum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

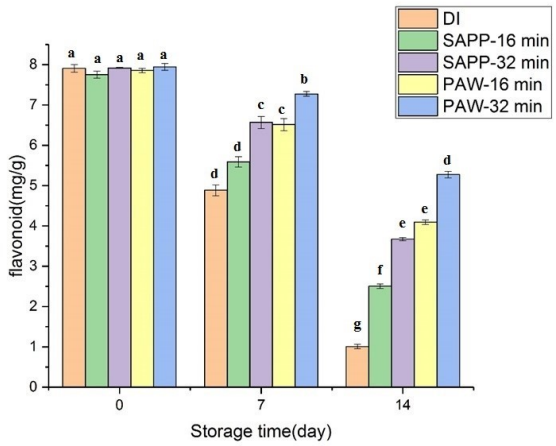

Figure 4. Changes in flavonoid content of P. crispum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

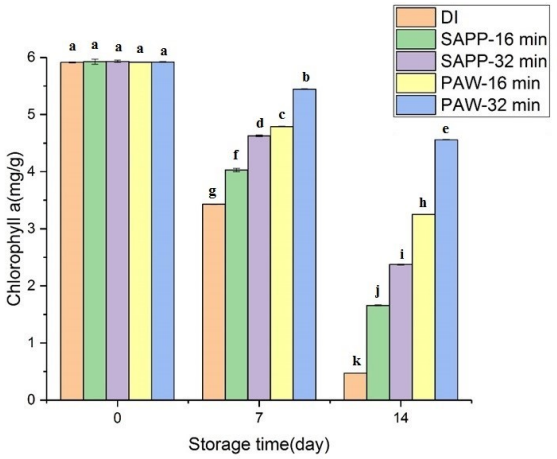

Figure 6. Changes in chlorophyll a content of P. crispum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

living tissues to eradicate pathogens, blood clotting, sterilization of multidrug-resistant bacteria, and the destruction of cancer cells [17–21]. In recent years, the use of atmospheric pressure plasma as a new and unique technology in the production of PAW, due to its effectiveness against a wide range of microorganisms without adversely affecting the quality of the product, for disinfection and storage of food and increasing the shelf life of fresh vegetables and fruits has been considered. PAW is generated by discharging the plasma into water or above its surface. Reactive species of nitrate, nitrite, and hydrogen peroxide are produced with long life and essential role in PAW [22–29]. Recent works have reported that PAW can effectively inactivate a wide range of bacteria and fungi in fresh vegetables such as fresh-cut celery and radicchio [30], fresh-cut iceberg lettuce and fresh mung bean sprouts [31], fresh-cut pears [32], fresh-cut apple [33], fresh-cut Endive lettuce [34], grapes [35, 36], lettuce [37], Chinese bayberries [38], fresh-cut endive lettuce [39], fresh-cut lettuce [40], spinach leaves [41] and fresh-cut kiwifruit [42]. The important point is that using PAW does not significantly change the quality of freshly sliced fruits [26, 32, 33, 35, 37, 42]. Therefore, PAW is a new technology to preserve and increase the shelf life of freshly cut fruits and vegetables. However, so far, fewer reports have been published on the effects of PAW on the antioxidant properties of freshly cut fruits [33]. In particular, no report has been published on the evaluation of phenolic content, flavonoid content, antioxidant activity, chlorophyll a, chlorophyll b, total chlorophyll content and carotenoids of PAW-washed vegetables and their effect on the shelf life of these materials over time. Specifically, there have been no reports of PAW being used to wash Petroselinum crispum (P. crispum) and Lepidium sativum (L. sativum) and investigate these cases. On the other hand, studies have been limited to the antibacterial and antifungal effects of PAW. In addition, there is no information on the effects of PAW

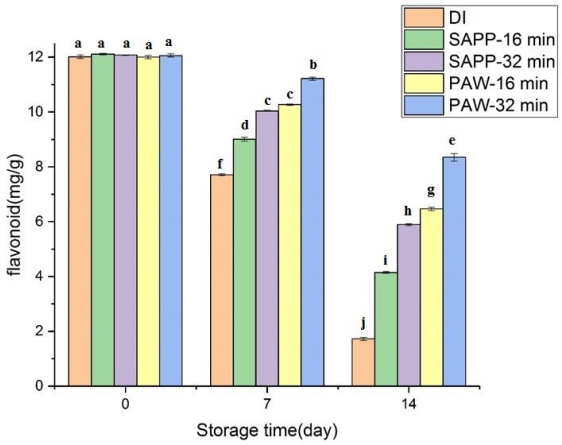

Figure 5. Changes in flavonoid content of L. sativum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

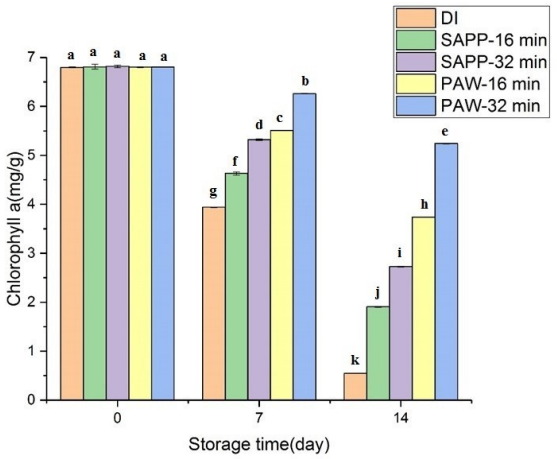

Figure 7. Changes in chlorophyll a content of L. sativum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

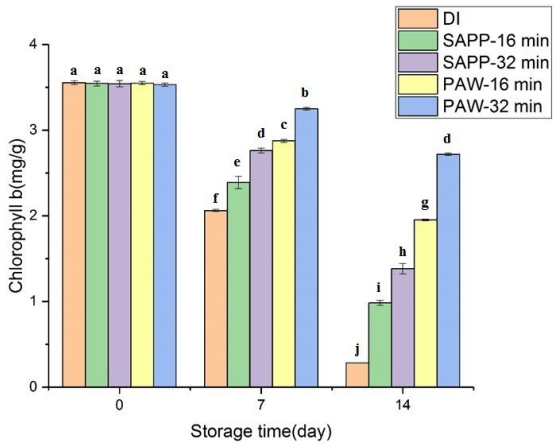

Figure 8. Changes in chlorophyll b content of P. crispum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

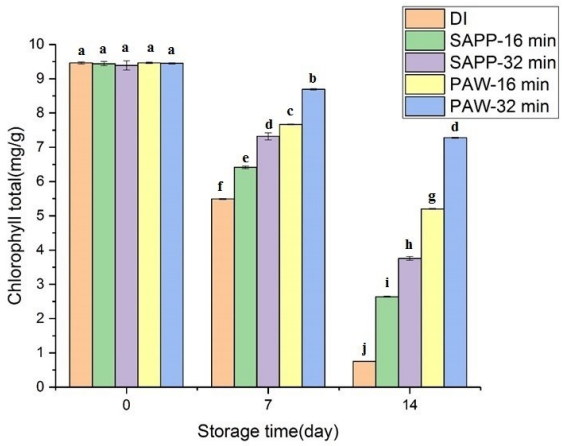

Figure 10. Changes in chlorophyll total content of P. crispum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

on the physicochemical, sensory, and nutritional properties of freshly cut fruits and vegetables.

The present paper aims to highlight plasma, PAW in particular, as a means of alternative, an innovative and unique method for its application in different aspects of food and vegetable processing and preservation. To investigate the effect of PAW on nutrient retention in vegetables, an experimental set was used to produce PAW. To produce PAW, DI was used in one case and 1% disodium pyrophosphate (SAPP) solution in the other. To compare the effect of DI and PAW and, the role of reactive species on plant nutrients, PAW was produced in two different modes, every 16 and 32 minutes. The concentrations of reactive species in two different states in PAW were calculated to compare their effect on plant nutrient retention. Then, the vegetables were washed with DI and PAW in two cases for 20 minutes. The samples were stored at 4 °C for 14 days. Immediately after washing and on the seventh and fourteenth days, levels of phenolic content, flavonoid content, antioxidant activity, chlorophylls and carotenoids were measured. The results showed that using PAW causes the nutrients to remain in the vegetables for a longer time. By comparing DI and PAW, we show that nutrient persistence in vegetables depends on the concentration of reactive species in PAW and the type of species.

2. Experimental section

2.1 Experimental setup

In this study, the experimental setup for transient spark discharge (TS) is depicted in Fig. 1. A needle electrode as a high voltage electrode (HV) and a hollow copper tube that allows us to inject nitrogen into the solution is intended as ground electrode that enables us to inject the discharge into the solution.

To determine the concentration of NO_3, NO_2, and H_2O_2 in PAW, UV absorption spectroscopy was performed and, the

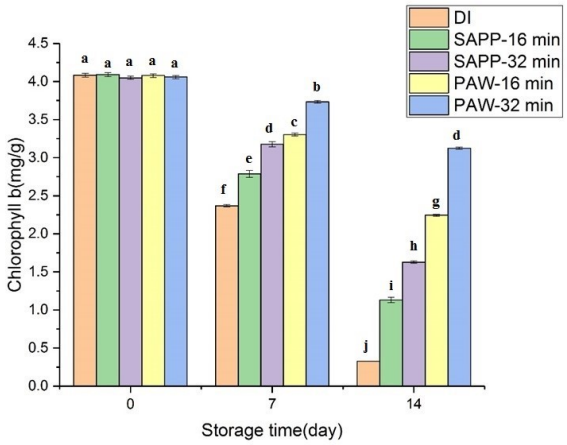

Figure 9. Changes in phenol content of L. sativum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

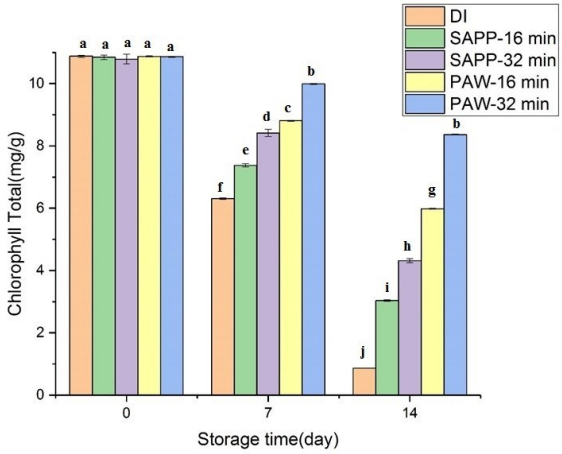

Figure 11. Changes in chlorophyll total content of L. sativum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

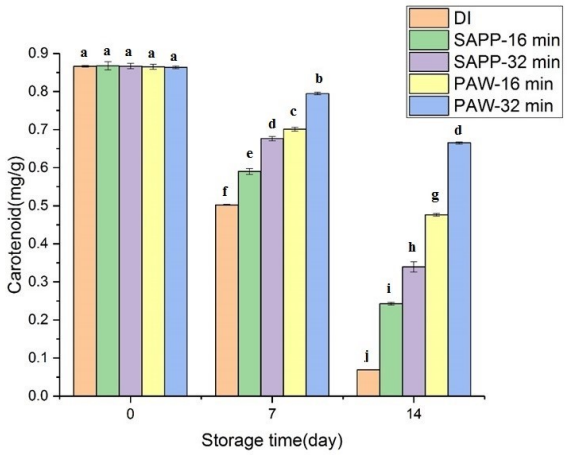

Figure 12. Changes in carotenoid content of P. crispum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

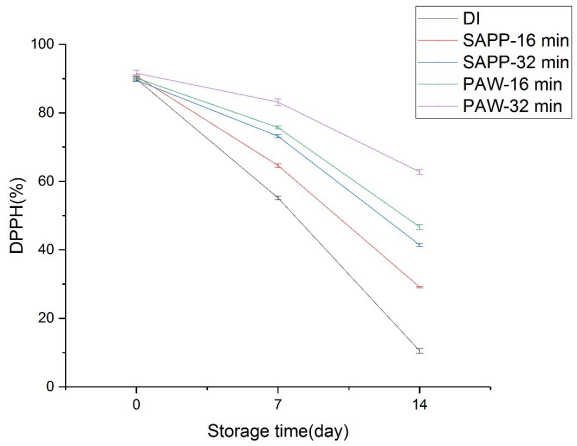

Figure 14. DPPH radical scavenging activities of P. crispum during storage at 4 °C for 14 days.

methods described by Liu et al. were used (Liu et al. 2019).

2.2 Processing and treatment of fresh vegetables with DI and PAW

Two types of fresh vegetables (P. crispum and L. sativum) were purchased from a local farm (Mazandaran, Iran). Vegetables with similar conditions and free from any fungal, bacterial, and physical damage were carefully selected. Fresh vegetables were stored in the laboratory for 24 hours at 4 °C for the same initial conditions. Then, 350 mL of DI and two samples of PAW were prepared for washing fresh vegetables. DI was used to produce PAW in one case and 1% SAPP solution in the other. Fresh vegetables were randomly immersed in DI and, two samples of PAW were prepared at 16 and 32 minutes. The experiments were repeated four times. After each treatment, the samples were transferred to zipper bags. The samples were transferred to a refrigerator at 4 °C and stored there for up to 14 days. The content of phenols, flavonoids, antioxidant activity, chlorophylls and carotenoids of the samples was evaluated on the first day after washing and on the seventh and fourteenth days.

2.3 Measurement of total phenolic and flavonoid content

To prepare the extract, 5 ml of 80% methanol was pounded in a mortar and pestle with 0.25 g of vegetables. The samples were then placed on an ultrasonic device for 10 minutes. Finally, the samples were centrifuged at 5000 rpm for 5 minutes and, the supernatant was separated. Phenols was assayed by Folin-Ciocalteu method. A volume of 200 μL of the extract and 1000 μL of 10% folin reagent were mixed and, after 5 minutes, 800 microliters of 7.5% sodium carbonate were added to it. After 60 minutes of remaining in the dark, the sample absorption was read at 765 nm. The amount of phenols was calculated using standard curve obtained from different concentrations of gallic acid and expressed in milligrams per gram of sample weight [43].

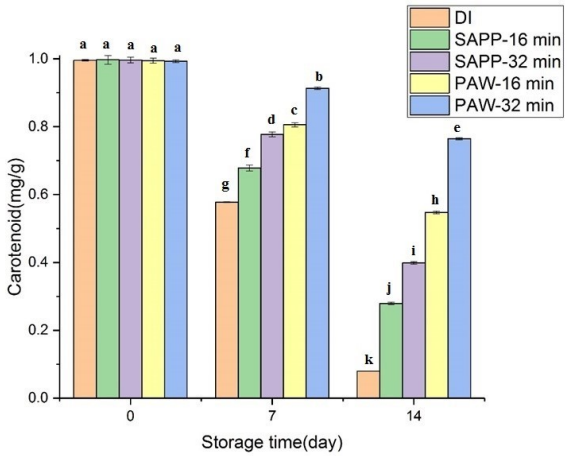

Figure 13. Changes in carotenoid content of L. sativum during storage at 4 °C for 14 days. Different letters indicate significant differences at the 5% level.

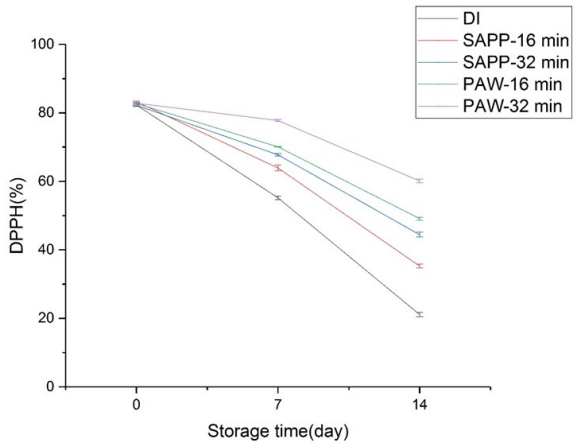

Figure 15. DPPH radical scavenging activities of L. sativum during storage at 4 °C for 14 days.

Table 1. NO_3, NO_2, and H_2O_2 concentrations when DI was used to produce PAW.

Time (min)	NO_3 Concentration (mg/L)	NO_2 Concentration (mg/L)	H_2O_2 Concentration (mg/L)
4	78.3±0.2	16.66±0.06	2.982±0.009
8	103.8±0.2	22.87±0.04	4.104±0.007
12	135.7±0.3	29.63±0.07	5.28±0.01
16	176.7±0.1	40.89±0.03	6.413±0.004
20	216.8±0.1	50.86±0.03	8.08±0.001
24	258.6±0.2	57.45±0.05	10.028±0.008
28	311.3±0.4	68.57±0.08	12.00±0.01
32	379.7±0.1	79.89±0.03	14.158±0.005

Flavonoids were assayed by the aluminum chloride colorimetric method. To 200 μL of the extract, 40 μL of 10% aluminum chloride, 40 μL of 1 M potassium acetate, 600 μL of 80% methanol and, 1120 μL of distilled water were added and, after 30 minutes, the samples were absorbed in a spectrophotometer at 415 nm. Finally, the amounts of flavonoids were expressed using standard curve of different concentrations in terms of mg/g sample weight [44].

2.4 Measurement of chlorophylls and carotenoids

To extract photosynthetic pigments, 5 ml of 80% acetone and 0.25 g of vegetables were pounded in a mortar and pestle. The samples were then placed on an ultrasonic device for 10 minutes. Finally, the samples were centrifuged at 5000 rpm for 5 minutes and, the supernatant was separated. Then the absorbance of the samples was read at 470, 645, and 663 nm. Finally, the amount of chlorophylls and carotenoids were calculated in terms of mg/g of the sample using the following formulas [45].

$$\text{chlorophyll a} = 12.7 \times A_{663} - 2.69 \times A_{645} \quad (1)$$

$$\text{chlorophyll b} = 22.9 \times A_{645} - 4.68 \times A_{663} \quad (2)$$

$$\text{chlorophyll}_{total} = 20.2 \times A_{645} + 8.02 \times A_{663} \quad (3)$$

$$\text{Carotenoids} = \frac{1000 \times A_{470}}{198} - \frac{1.82 \times \text{chlorophyll a} - 85.2 \times \text{chlorophyll b}}{198} \quad (4)$$

where A_{470} is the absorbance of the sample on 470 nm, A_{645} is the absorbance of the sample on 645 nm, A_{663} is the absorbance of the sample on 663 nm.

2.5 Antioxidant capacity assays

To prepare the extract, 5 ml of 80% methanol and 0.25 g of vegetables were pounded in a mortar and pestle. The samples were then placed on an ultrasonic device for 10 minutes. Finally, the samples were centrifuged at 5000 rpm for 5 minutes and, the supernatant was separated. To measure the antioxidant activity, a volume of 200 μL of the extract, 800 μL of 80% methanol, and 1000 μL of DPPH were mixed, and after standing in the dark for 30 minutes, the absorbance of the samples at 517 nm was read. Finally, the amount of antioxidant capacity was calculated as a percentage of the following formula [46]. I = antioxidant capacity assays.
A_0 = control absorption rate.
$A - s$ = sample absorption rate.

$$I = \frac{A_0 - A_s}{A_0} \times 100 \quad (5)$$

Table 2. NO_3, NO_2, and H_2O_2 concentrations when 1% SAPP solution was used to produce PAW.

Time (min)	NO_3 Concentration (mg/L)	NO_2 Concentration (mg/L)	H_2O_2 Concentration (mg/L)
4	17.98±0.05	81.6±0.1	3.120±0.002
8	23.89±0.04	106.1±0.2	3.951±0.008
12	30.81±0.07	137.5±0.3	5.19±0.01
16	39.43±0.03	179.6±0.1	6.598±0.004
20	47.18±0.05	215.5±0.1	8.096±0.001
24	56.49±0.05	257.6±0.2	9.459±0.007
28	67.61±0.07	309.2±0.3	10.96±0.01
32	81.94±0.03	373.1±0.1	13.617±0.004

2.6 Statistical analyses

The data were analyzed using SPSS22 software. Statistical analysis was performed by one-way analysis of variance (ANOVA). When significant differences were detected, the differences among the mean values were determined by Duncan's multiple comparison test at a confidence level of $p < 0.05$. Mean values and standard errors of the mean are reported.

3. Results and discussion

3.1 Generation of RONS in the PAW

Evaluation of the induced concentration of RONS in solution is necessary to determine which of them can have a more significant impact on increasing the shelf life of the nutrients described above. As shown in the Table.1, when DI was used to produce PAW, the levels of NO_3 in treated samples were significantly higher than the other species. The increasing rate of the NO_3 concentration during the treatment time was faster than other species. The NO_3 concentration after plasma treatment for 4 minutes was 78.3±0.2 mg/L, which had risen to 379.7±0.1 mg/L with a treatment time of 32 minutes. The molar fraction of nitrate to nitrite in this sample was estimated to be equal to 4.32 after 16 minutes and equal to 4.75 after 32 minutes. In the case of the other sample, when 1% SAPP solution was used, as shown in the Table.2, the levels of NO_2 in treated samples were significantly higher than in the other species. The increasing rate of the NO_2 concentration during the treatment time was faster than other species. The NO_2 concentration after plasma treatment for 4 minutes was 81.6±0.1 mg/L, which had risen to 373.1±0.1 mg/L with a treatment time of 32 minutes. The molar fraction of nitrate to nitrite in this sample was estimated to be equal to 0.22 after 16 minutes and equal to 0.22 after 32 minutes.

Evaluation of the concentrations of the produced species showed that where DI was used, the concentration of nitrate was higher than all species, followed by the concentration of nitrite and then the concentration of hydrogen peroxide. When 1% SAPP solution was used to produce PAW, the nitrite concentration was higher than all species, followed by the nitrate concentration and then the hydrogen peroxide concentration. However, an increase in the concentration was observed during the treatment periods in both cases.

3.2 Influence of DI and PAW treatments on P. crispum and L. sativum

To evaluate the effect of PAW on the shelf life of nutrients, it is necessary to assess their amount when washed with DI and PAW. For this aim, the main characteristics of our samples, including the content of phenols, flavonoids, chlorophylls and carotenoids after washing with DI, and two samples of PAW at 16 and 32 minutes, after washing and seventh and fourteenth days after washing were profiled and measured. The results show that there is an overall declining trend in nutrient content for samples washed with DI and PAW during the entire storage period. When DI was used for washing, the amounts of nutrients contained in the samples were significantly reduced. The rate of reduction of nutrients contained in vegetables was very significant. When 1% SAPP solution was used to produce PAW and then used for washing, the rate of reduction of nutrients contained in the samples was slower than before.

Finally, when DI was used to produce PAW and then used for washing, the rate of reduction of nutrients contained in the samples was slower than in the previous cases. Therefore, it can be concluded that the presence of reactive species in PAW increases the shelf life of nutrients. PAW was produced two times for 16 and 32 minutes and used for washing. An important point to note is that as the plasma exposure time of the sample increases from 16 to 32 minutes, the rate of reduction of nutrients contained in the samples was slower than before.

In the following, the results of the effect of DI and PAW treatments on nutrient content in P. crispum and L. sativum are presented in detail and discussed. Phenolic compounds are a large group of natural plant substances, including phenols, flavonoids, tannins, etc., which are usually found in fruits, vegetables, leaves, nuts, seeds, roots, and other plant parts. Materials have significant benefits in the fields of food, chemistry, pharmaceuticals and medicine due to a wide range of favorable biological effects, including antioxidant properties [47–49]. The influences of DI and PAW treatments on phenolic content and flavonoid content on P. crispum and L. sativum are presented in Figs. 2 to 5.

As shown in Figs. 2 and 3, the initial amount of phenolic content in P. crispum and L. sativum in all samples was about 5.31 and 7.94 mg/l, respectively. When DI was used for washing, the amount of phenolic content in the samples was significantly reduced. After 7 days, the amount of phenolic content in P. crispum and L. sativum decreased to 60.15 and 57.09% of the initial value, respectively, and after 14 days, it decreased to 10.63 and 7.85% of the initial value, respectively. When 1% SAPP solution was used to produce PAW and then used for washing, the rate of reduction of phenolic content in the samples was slower than before. For PAW in this state for 16 minutes, After 7 days, the amount of phenolic content in P. crispum and L. sativum decreased to 70.11 and 68.99% of the initial value, respectively, and after 14 days, it decreased to 29.70 and 27.45% of the initial value, respectively. For PAW in this state for 32 minutes, After 7 days, the amount of phenolic content in P. crispum and L. sativum decreased to 77.38 and 78.97% of the initial value, respectively, and after 14 days, it decreased to 46.17 and 43.68% of the initial value, respectively. Finally, when DI was used to produce PAW and then used for washing, the rate of reduction of phenolic content in the samples was slower than in the previous cases. For PAW in this state for 16 minutes, After 7 days, the amount of phenolic content in P. crispum and L. sativum decreased to 80.30 and 81.27% of the initial value, respectively, and after 14 days, it decreased to 48.86, and 56.05% of the initial value, respectively. For PAW in this state for 32 minutes, After 7 days, the amount of phenolic content in P. crispum and L. sativum decreased to 89.27 and 91.91% of the initial value, respectively, and after 14 days, it decreased to 64.78 and 77.15% of the initial value, respectively. The results show that the greatest reduction in phenolic content is when deionized water is used for washing and, the best performance is when DI is

used to produce PAW in 32 minutes.
As can be seen in the diagrams, by examining the results obtained for flavonoid content, it can be seen that this general trend for them is similar to that of phenolic content.
Chlorophyll is the green pigment found in plants that helps absorb sunlight and convert it into energy. It is believed that this substance is very useful for the human body. Consuming foods containing chlorophyll cleans the blood flow, eliminates bad breath and body odor, neutralizes carcinogens and prevents tooth decay. Chlorophyll, as a strong antioxidant agent, reduces the destruction of cells by environmental carcinogens [48, 49]. The influences of DI and PAW treatments on chlorophylls on P. crispum and L. sativum are presented in Figs. 6 to 11.
The results for Chlorophylls show that the use of PAW increase the retention time of them in the samples and the rate of reduction in the samples also takes place with a lower slope.
Carotenoids are a group of pigments that, in addition to their role in the formation of pigments, antioxidant properties have also been reported for them. Animals and humans do not have the ability to synthesize them, and they must be received through the diet, after which they can be converted from carotenoid form to another form. They also play an important role in the formation of vitamin A [47–49]. The influences of DI and PAW treatments on carotenoids on P. crispum and L. sativum are presented in Figs. 12 and 13.

3.3 Influences of DI and PAW treatments on antioxidant activity

The antioxidant activities of P. crispum and L. sativum, assessed by DPPH assay, are shown in Fig. 14 and 15. An overall declining trend in DPPH radical scavenging activity was observed for DI and PAW-washed samples during the entire storage period. When PAW was used for washing, it exhibited the best retention of DPPH radical scavenging activity during the entire 14-day storage period. When DI was used for washing, the DPPH radical scavenging activity in the samples was significantly reduced. The rate of reduction of nutrients contained in vegetables was very significant. After fourteen days, for P. crispum, it increased from 89.87% to 10.53%, and for *L.*
sativum, from 82.28% to 21.13%. When 1% SAPP solution was used to produce PAW and then used for washing, the rate of reduction of DPPH radical scavenging activity in the samples was slower than before. After fourteen days, for P. crispum, in the best case, it increased from 89.53% to 41.043%, and for L. sativum, from 83.23% to 44.46%. Finally, when DI was used to produce PAW and then used for washing, the rate of reduction of DPPH radical scavenging activity in the samples was slower than in the previous cases. After fourteen days, for P. crispum, in the best case, it increased from 91.6% to 62.78%, and for L. sativum, from 82.79% to 60.10%.
In few researchs the effect of cold plasma on the antioxidant activity of fruit was measured. Their results showed that the fruit antioxidant content did not significant changes [47–50]. The results of our research also showed that the use of PAW increases the shelf life of antioxidant activity for a longer period. Therefore, using water for washing can be an effective way to improve the quality of food properties in the long term.

4. Conclusion

In conclusion, the effect of the two samples of PAW and DI on the P. crispum and L. sativum was investigated. It was concluded that using PAW compared to DI for washing increases the shelf life of nutrients in fresh vegetables. On the other hand, by comparing two samples of activated water produced, it is determined that nitrate is more effective than nitrite. Also, increasing the production time of PAW from 16 to 32 by increasing the concentration of the species plays an important role in the retention of nutrients in fresh vegetables. In summary, our results and findings clearly show that our innovative vegetable washing strategy can effectively retain nutrients in vegetables for longer periods. While minimizing damage to the quality of vegetables, and no harmful substances are added to them. In the future, studies and research should focus on proposing optimal, low-cost, and scalable plasma methods or devices for widespread applications in the food industry. The results of this study could be used in plasma-based vegetables processing technology for packaging and maintaining.

Conflict of interest statement:
The authors declare that they have no conflict of interest.

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