Research article

Preprocess Sonication Maintains Quality and Inhibits Browning of Fresh-cut Fruit Using Apples as the Fruit Model

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Keywords

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Abstract

Ultrasonic (US) waves (sound waves) have been used to preserve and improve the quality of fresh fruits and vegetables. The goal of this study was to determine whether pre-process US treatment was useful in reducing browning and retaining certain aspects of fresh-cut 'Gala' apples' quality. The apples were sonicated at a frequency of 120 kHz for 0, 5, 10 and 15 min before processing and then the processed apples were stored at 4±1°C. The US treatment at 5 min significantly delayed the loss of lightness, and increases in colour difference and browning index, and maintained whiteness compared to other treatments. The US treatment at 5 min controlled enzymatic browning reaction, probably due to inhibition of polyphenol oxidase activity. US treatments could delay the loss of hardness compared to the control treatment but had no influence on the total soluble solids content. Interestingly, US treatments induced antioxidant activity in fresh-cut apples during storage. In summary, the 5 min sonication could prevent browning and enhance the quality of fresh-cut apples during storage.

1. Introduction

The fresh-cut fruit and vegetable market size has expanded rapidly in recent years. Fresh-cut products have been claimed as convenient and healthy food products that can meet the modern lifestyle and health-conscious consumers. However, fresh-cut products are commonly acknowledged to be extremely perishable due to physiological changes, degradation, and microbial growth. Refrigeration is mandatory to preserve the quality and safety of fresh-cut products. To prevent physiological changes and maintain the quality of fresh-cut products, a storage temperature

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of approximately 4°C is advised. Even while fresh-cut fruit and vegetables are effectively protected from degradation and microbial growth by refrigeration, certain issues including browning, discoloration, and softening still occur [1, 2]. Fresh-cut apples are a kind of minimally processed fruit that has a big market share. As the consumption of fresh-cut fruit and vegetables has been advised, fresh cut apples make up a sizeable portion of the food markets around the world [1]. Browning incidence is recognized as a primary issue impacting the shelf-life and acceptability of fresh-cut fruit and vegetables, especially fresh-cut apples. Enzymatic browning is generally recognized as a sign of deterioration and is linked to undesirable change in the color and flavor of fresh-cut products. Polyphenol oxidase (PPO) and polyphenol peroxidase (POD) are known to have roles in the process of browning in fresh-cut products by catalyzing the oxidation of free radicals and phenolic substances [2].

In order to maintain freshness and prevent discoloration of fresh-cut fruit and vegetables, anti-browning and texture-preserving compounds are utilized on an industrial scale [2, 3]. Recently, the application of chemicals in food products has concerned consumers. Since browning affects the fresh-liked quality of fresh-cut apples, we were interested in finding a physical approach to avoid it. Ultrasonics (US) is recognized as a physical elicitor used to preserve and maintain the physicochemical quality and degrade hazardous chemical residues in fresh fruit and vegetables. US causes temporary acoustic cavitation and microstream effects that remove hazardous chemical residues and microorganisms from surfaces and enhance defense mechanisms as well as antioxidant systems in plants [4-6]. However, excessive doses of ultrasonics degrade plants by causing tissue disruption [6]. According to Wen *et al*. [7], using post-cut ultrasonic at 40-kHz frequency for 2 min reduced the browning incidence in lotus root slices for 6 days and also enhanced the firmness. Pan *et al*. [8] suggested that enzymatic browning of fresh-cut sweet potatoes was inhibited by the use of ultrasound at 40-kHz frequency for 2 min. Moreover, the treatment also enhanced antioxidant capacity and antioxidant enzyme activities in the sweet potato slices. According to Jang *et al*. [9], post-cut ultrasonic treatment at 40-kHz for 1 min prevented the enzymatic browning response in apple slices. The efficacy of the browning inhibition was enhanced when combined with 1% ascorbic acid. These studies demonstrated that US treatment is a feasible approach for preserving and/or improving the quality of fresh-cut products.

Therefore, we became interested in using ultrasonic preprocessing treatment to control the incidence of enzymatic browning and maintain the physicochemical quality attributes of fresh-cut apples using ‘Gala’ apple as the fruit model.

2. Materials and Methods

2.1 Raw materials and treatments

‘Gala’ apples (*Malus domestica*) were purchased from a retail fruit market named Talad Thai. The fruits had been screened for damage and the peduncles were fresh. After being cleaned twice with tap water, the fruits were separated into 4 groups (17 fruits per group). The first group was untreated (control). The second, third and fourth groups were subjected to sonication for 5, 10 and 15 min, respectively, at a frequency of 120 kHz using a 3L digial ultrasonic cleaner (Shanghai Peixu Machinery Equipment Co. Ltd., China). The apples were then halved vertically without removing the peel. The core was then removed after each half had been separated into four equal parts. Eight apple wedges were placed in a 5 x 5 inch polyethylene terephthalate clamshell box. The samples were kept at 4±1°C for 7 days. Four boxes of each treatment (4 replicates per treatment) were separated to determine visual appearance and superficial color values every day. On days 0, 3 and 7

of storage, the remaining four boxes of each treatment were chosen at random in order to assay the antioxidant activity, total phenol content, texture, and browning enzyme activity.

2.2 Determining factors for appearance and color

Photographs were used to assess how the fresh-cut apples' appearance changed. A Hunter colorimeter, MiniScan 4500 (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used to assess the L^*, a^*, and b^* values of cut-surface samples. The color difference value (ΔE^*) was calculated as follows: $\Delta E^* = [(L_0^* - L_x^*)^2 + (a_0^* - a_x^*)^2 + (b_0^* - b_x^*)^2]^{1/2}$, where 0 = initial day and x = sampling date. The whiteness index (WI) (1) and browning index (BI) (2) values [7] were calculated.

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (1)$$

$$BI = 100 \times (X - 0.31) / 0.172 \quad (2)$$

where $X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$

2.3 Texture and total soluble solids measurements

A Texture Analyzer, EZ-SX (Stable Micro Systems, USA), was used to measure apple wedge hardness. The penetration force of the middle of an apple wedge was measured using a cylinder-shaped probe with a diameter of 6.0 mm. The sample's hardness in newtons (N) was recorded as the measurement's maximum value. The total soluble solids content of the juice extracted from the sample was assessed using a hand-held refractometer model 2200 H-50 (Atago Co. Ltd., Japan).

2.4 The assays of polyphenol oxidase (PPO) and peroxidase (POD) activities

At a temperature below 4°C, 0.1 mol L^{-1} sodium phosphate buffer pH 7.0 containing polyvinylpyrrolidone (PVPP) was used to extract the sample. The method developed by Galeazzi *et al*. [10] was used to determine PPO activity. The PPO activity was measured as a 0.001 change in absorbance at 420 nm wavelength per minute and the activity was displayed as units per kg of fresh weight (U g^{-1}). The POD activity was assessed following the procedure given by Cuvi *et al*. [11]. The activity of POD was identified via a rise in absorbance at 470 nm wavelength per minute, and the enzyme activity was recorded in units per kilogram of fresh weight (U g^{-1}).

2.5 Total phenolic compounds concentration assay

Total phenolic compounds of the fresh pulp (3 g) were determined using the method of Slinkard and Singleton [12]. The absorbance at 750 nm wavelength was recorded and the total amount of phenolic components was obtained using a linear equation generated from a gallic acid standard curve. The concentration was given as µg gallic acid equivalent per gram of fresh weight (µg g^{-1}).

2.6 Antioxidant activity assay

Using the same extract from the total phenolic compound concentration assay, the samples' antioxidant activity was evaluated. The ferric reducing antioxidant potential (FRAP) method, invented by Benzie and Strain [13], was used to assay the antioxidant activity. The trolox standard

solution's linear equation was used to calculate the antioxidant activity, and the results were expressed as micromoles of trolox equivalent per gram of fresh weight (μmol g^{-1}).

2.7 Statistical analysis

This study used a completely randomized design (CRD). There were four replicates of each physicochemical parameter measurement. Using the SPSS program (SPSS Inc., Chicago, IL, USA), the difference between each treatment was evaluated by analysis of variance (ANOVA) and the means were compared using a Post Hoc Duncan's new multiple range test at a significance level of P value<0.05.

3. Results and Discussion

3.1 Superficial color attributes

The changes in L^* (brightness), ΔE^*, WI and BI values of the apple wedges during storage are depicted in Figure 1. After being stored for 1 day, decreases in L^* and WI values and increases in ΔE^* and BI values were observed. The L^* value of samples that were sonicated for 5 min was higher than those of other treatments during storage. After being stored for 7 days, the samples that were sonicated for 5 min had L^* values that were noticeably greater than those of other samples ($P<0.05$), although samples that were sonicated for 0, 10, and 15 min had similar L^* values. The ΔE^* values of all treatments continuously increased throughout the storage period. The lowest increase in ΔE^* value was observed for the 5 min sonicated samples, while the increased ΔE^* values of other treatments were likely to be similar. All samples had a marked decline in WI value after being stored for 1 day. In comparison to the control and 15 min sonicated samples, the WI value of the 5 min sonicated samples was significantly greater ($P<0.05$). Compared to the 10 min sonicated samples, the WI values of samples that were sonicated for 5 min were likely to be higher. After being kept for 1 day, BI values in all treatments showed a noticeable rise. Following that, the 5 min sonicated samples showed the lowest rise in BI value, which was significantly lower than other treatments ($P<0.05$). The increase in BI value of the 10 min sonicated samples tended to be higher than those of other treatments during storage. The 5 min sonicated samples were clearly seen to have significantly greater L^* and WI values and lower ΔE^* and BI values than other treatments on day 7 of storage. This was in line with earlier studies showing that ultrasonic treatment could prevent discoloration, especially browning incidence, in lotus root slices [7] and that the application of ultrasonic cutting preserved the color of fresh-cut apples [14]. Additionally, according to Pan *et al.* [8], ultrasonic treatment at 40 kHz for 10 min prevented color change and inhibited the increased browning of fresh-cut sweet potatoes. The previous work also addressed the fact that controlling the enzymatic browning reaction was related to the suppression of discoloration of fresh-cut products by ultrasonic treatment.

3.2 Browning enzyme activities and total phenolic compounds

Figures 2 and 3 display the activities of the browning enzymes, PPO and POD, and the total phenolic compounds of the fresh-cut apples that were preprocess-sonicated for 0 (control), 5, 10 and 15 min. After treatment, the PPO activities of all sonicated samples were significantly lower than that of the control ($P<0.05$) (Figure 2A). The 5 min sonicated samples had the lowest PPO activities on days 3

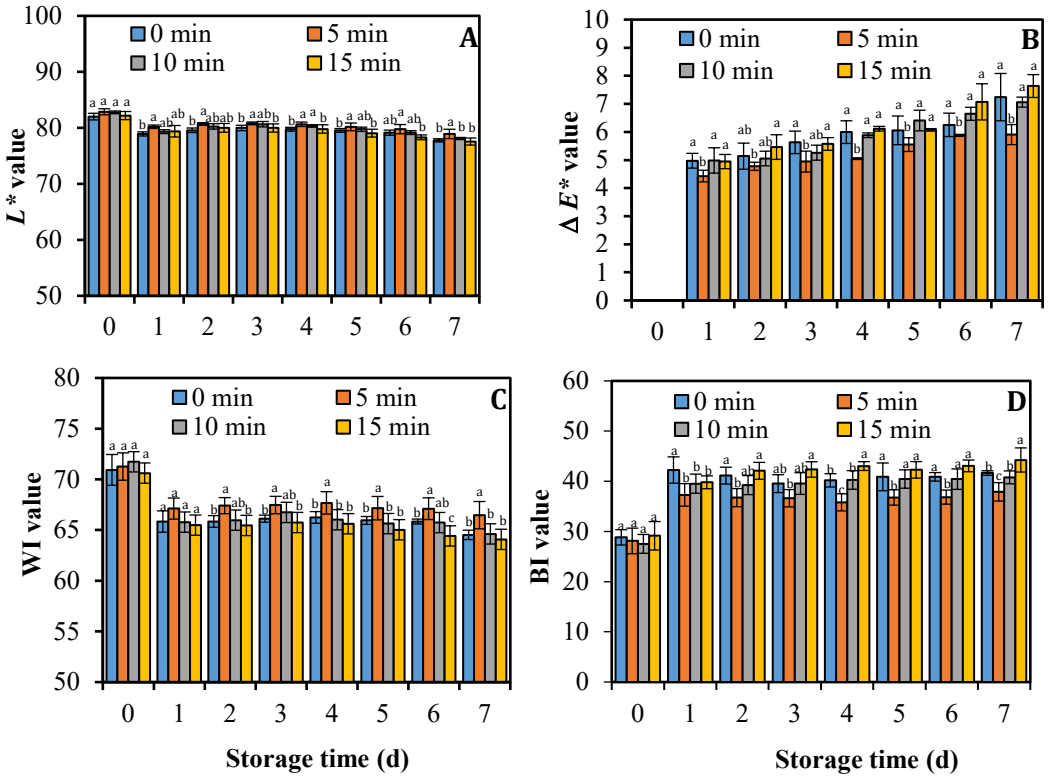

Figure 1. Superficial color attribute values, L^* (A), ΔE^* (B), WI (C) and BI (D), of 0 to 15 min preprocess-sonicated fresh cut apples during storage at 4+1°C for 7 days. The means of four replications with a vertical bar of standard deviation are presented and the different letters designate the differences between the treatments on the same day.

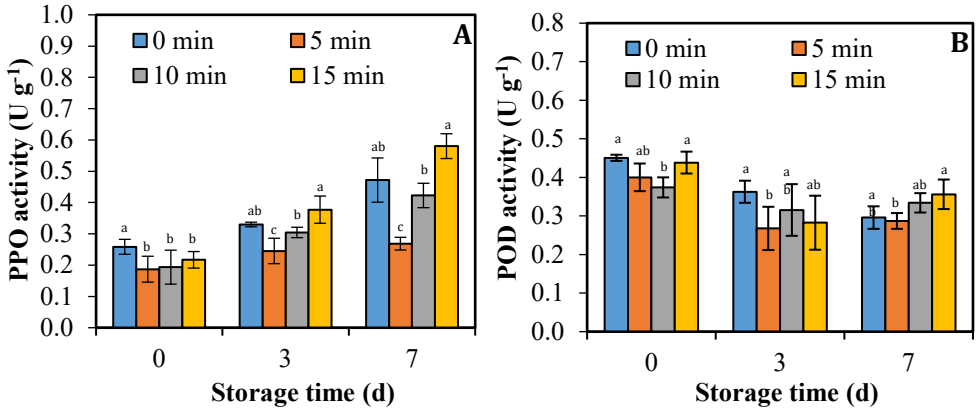

Figure 2. The activities of polyphenol oxidase (PPO) (A) and peroxidase (POD) (B) of 0 to 15 min preprocess-sonicated fresh cut apple during storage at 4+1°C for 7 days. The means of four replications with a vertical bar of standard deviation are presented and different letters designate the differences between the treatments on the same day.

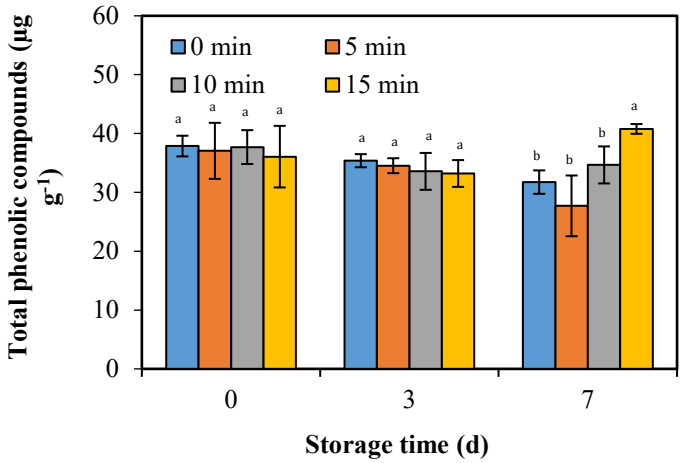

Figure 3. The total phenolic compounds contents of fresh-cut apples that were preprocess-sonicated for 0, 5, 10 and 15 min during storage at 4±1°C for 7 days. The means of four replications with a vertical bar of standard deviation are presented and the different letters designate the differences between the treatments on the same day.

and 7 of storage, which were considerably lower than those of other treatments ($P<0.05$). The increased PPO activities of the control and the 15 min sonicated samples were likely to be similar and were higher than those of the 10 min and 5 min sonicated samples, respectively. In comparison to the control, the POD activities of the 5 and 10 min sonicated samples were significantly reduced after treatment ($P<0.05$) (Figure 2B). The POD activity of the 15 min sonicated samples, however, was comparable to that of the control samples. The 5 min sonicated samples had the lowest POD activity on day 3 of storage, which was significantly different from only the control samples ($P<0.05$). The samples that had been sonicated for 15 min had the highest POD activity after being kept for 7 days; this activity was significantly different from the samples that had been sonicated for 5 min ($P<0.05$). The POD activity of the samples that were sonicated for 5 min was not different from those of the control and 10 min samples. After storage for 3 days, there was no discernible variation in the total phenol concentrations of any treatment (Figure 3). The 15 min sonicated samples had the greatest total phenol concentration on day 7 of storage, which was significantly different from other treatments ($P<0.05$). While no discernible difference was found between the control, 5 min, and 10 min sonicated samples. The PPO enzyme's interaction with phenolic substances as substrates widely considered to be the primary cause of the enzymatic browning in fresh-cut apples [14, 15]. This study clearly demonstrated that PPO activity had a stronger impact on the browning of the fresh-cut apples than the POD activity. The ultrasonic treatment at an appropriate period inhibited the increase of PPO activity, and the preprocessing ultrasonic treatment at 120 kHz for 5 min effectively inhibited the increase of PPO activity in this study. Moreover, we also found that after being stored for 3 days, the POD activity of all ultrasonic treated samples was likely to be lower than that of the control samples. Cao *et al*. [16] , Jiang *et al*. [17] and Wen *et al*. [7] suggested that the cavitation and microstreaming effects of sonication might affect protein structure, resulting in the inactivation of enzyme activities. In a similar line, Pan *et al*. [8] also observed that increases in PPO and POD activities in fresh-cut sweet potatoes were suppressed by the post-cut ultrasonic treatment at 40 kHz for 10 min. Curiously, we discovered that the ultrasonic treatment for 15 min induced PPO and POD activities. In plants, phenolic compounds are typically housed in vacuoles that are bounded by a tonoplast, a lipoprotein membrane. POD and PPO enzymes

have been found in a range of membrane-bound cell organelles such as thylakoids, mitochondria and peroxisomes, and also in the soluble portion of the cell [10]. The disruption of the cell membrane and the increased release of membrane-bound enzymes and phenolic compounds caused by the excessive cavitation and microstreaming effects of sonication might be responsible for the high activity of both enzymes and the total phenolic compound content. Therefore, the rise in total phenolic compounds seen in the fresh-cut apple treated with sonication for 15 min after storage for 7 days led us to hypothesize that tissue rupture produced by excessive ultrasonic treatment would reduce insoluble bound phenolic compounds and increase soluble phenolic compounds.

3.3 Texture and soluble solids content

The effects of preprocess sonication on texture and total soluble solids content of fresh-cut apples are depicted in Figure 4. The hardness of the control samples tended to decrease during storage compared to the preprocess-sonicated samples. Fresh-cut apples that were sonicated for 15 min had a tendency to be harder than the samples treated in other treatments. The sonication treatment had no impact on the total soluble solids content of the fresh-cut apples during storage. Throughout storage, the total soluble solids content of the fresh-cut apples remained constant. Similar to this, earlier research showed that US treatment had no impact on the amount of soluble solids in guavas [4, 18]. It was claimed that ethylene produced by wounds accelerates the softening process and flavor loss in fresh-cut products [19]. Jiang *et al*. [17] reported that the biosythesis of ethylene (C_2H_4) could be controlled by sonication. Previous studies reported that the texture of lotus root slices [7], guavas [4] and fresh-cut cucumbers [20] could be maintained by the use of ultrasonic treatment. Liu *et al*. [21] and Jiang *et al*. [17] demonstrated that ultrasonic treatment inhibited the increases in cell wall hydrolase activities such as polygalacturonase, β-galactosidase and pectin methylesterase. Additionally, the application of ultrasonic treatment causes an increase in intracellular Ca^{2+}, which can then bind to de-esterified pectin polymers to form the calcium pectate's egg-box structure [4] [7]. An earlier study also suggested that cell wall polysaccharides can become more stable after being subjected to ultrasonic treatment [17].

3.4 Antioxidant activity

Figure 5 illustrates the effect of preprocess ultrasonic treatments on the antioxidant activity of the fresh-cut apples during storage. Antioxidant activity did not differ between the treatments during the 3 days of storage and was likely to remain constant. The antioxidant activity in the control samples was found to be considerably lower than those of all the preprocess-sonicated samples after being kept for 7 days ($P<0.05$); whereas all preprocess-sonicated samples had comparable antioxidant activities. Numerous earlier studies demonstrated that ultrasonic treatment enhanced antioxidant systems and secondary metabolite synthesis in plants through the induction of phenylpropanoid pathways [5, 8, 19]. US tereatment improved antioxidant defense, including the activity of antioxidant enzymes such superoxide dismutase and catalase, according to Huang *et al*. [22]. Khademi *et al*. [23] also reported that stress response due to US treatment increased antioxidant defense mechanisms, leading to a tolerance for oxidative stress and maintaining the quality of bananas. In a similar vien, antioxidant activity enhancement by US treatment was reported for lotus root slices [7], guavas [4, 18], fresh-cut apples [14] and fresh-cut sweet potatoes.

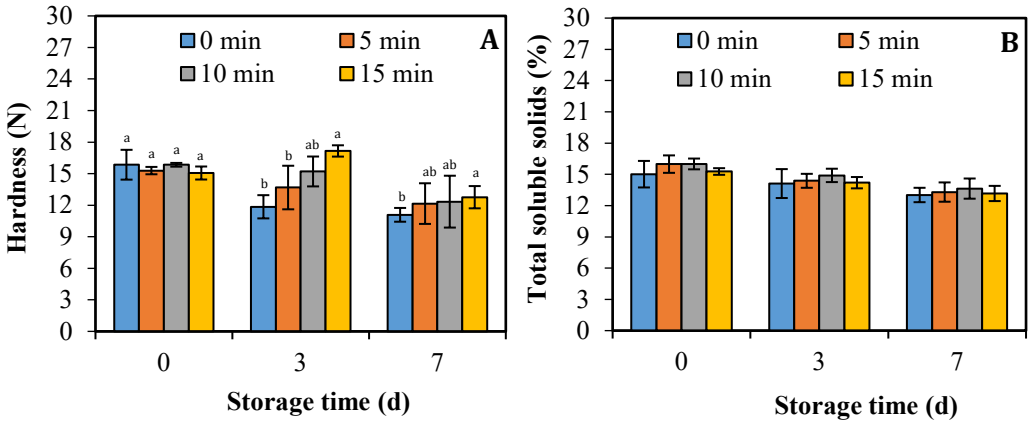

Figure 4. The texture (hardness) (A) and total soluble solid content (B) of 0 to 15 min preprocess-sonicated fresh cut apple during storage at 4+1°C for 7 days. The means of four replications with a vertical bar of standard deviation are presented and the different letters designate the differences between the treatments on the same day.

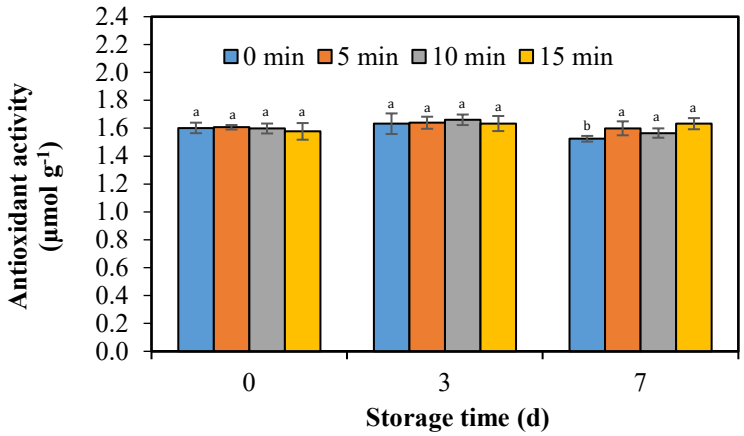

Figure 5. The antioxidant activity of 0 to 15 min preprocess-sonicated fresh cut apple during storage at 4+1 °C for 7 days. The means of four replications with a vertical bar of standard deviation are presented and the different letters designate the differences between the treatments on the same day.

4. Conclusions

An appropriate timing of preprocess ultrasonic treatment reduced the browning severity of fresh cut apples during cold storage. Subjecting the fresh-cut apples to ultrasonic treatment at a frequency of 120 KHz for 5 min prevented BI and color change (ΔE^*) while preserving lightness (L^*), whiteness (WI), and firmness. This treatment decreased PPO activity and increased antioxidant activity in apple slices at the end of storage. These results suggest that an appropriate approach for preserving quality and preventing discoloration in fresh-cut apples is preprocessed ultrasonic treatment at 120 kHz for 5 min.

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