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Inhibitory Effects of Ultrasound in Combination with Ascorbic Acid on Browning and Polyphenol Oxidase Activity of Fresh-cut Apples

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Abstract This study was conducted to investigate the effect of ultrasound with ascorbic acid on the quality of fresh-cut apples. Prepared apple cubes were dipped in distilled water (US) or in 1% ascorbic acid solution (AS), both were treated with ultrasound at 40 kHz, while the other sample was just dipped in 1% ascorbic acid solution for 1 min (AA). All samples were stored at 4°C for 12 days. AS treatment had an effect on apple cube color as indicated by its significantly higher L values and lower ΔE value whereas AA- and US-treated samples showed a considerable browning. Higher inhibition rate on browning and polyphenol oxidase (PPO) activity of 46 and 98%, respectively, were observed after AS treatment. The highest total phenolic content of AS-treated samples was shown during whole storage periods. This investigation revealed that the use of ultrasound in combination with ascorbic acid had a positive effect on quality maintaining of fresh-cut apples.

Keywords: ultrasound, antibrowning, polyphenol oxidase, non-thermal processing, 'Fuji' apple

Introduction

Recently, the food market is experiencing an increasing demand for fresh-cut fruits and vegetables. Fresh-cut produces have been more popular to consumers because of their convenient form, fresh-like quality, and health benefits thus the food industry has focused on the development of advanced processing techniques for prolonging shelf-life of fresh-cut produces (1-3).

Enzymatic browning in fresh fruits results from the oxidation of polyphenols to *O*-quinones catalyzed by polyphenol oxidase (PPO; EC 1.10.3.1), which react nonenzymatically with polyphenols and produce brown pigments. This discoloration is one of quality deterioration in fresh-cut fruits and vegetables (4,5). Producing fresh-cut products is accompanied by cell disruption of the fruit or vegetable flesh by peeling, coring, and slicing during processing. Once the cell structure is destroyed, browning would ensue from mixed enzyme and substrate. This discoloration of the cut surfaces is one of the most restrictive factors for the shelf-life of fresh-cut fruits and vegetables, especially sliced apples, and is the main hurdle for the development and commercialization of fresh-cut produces (4).

Ascorbic acid has been most widely used as an antibrowning agent for a long time and we can find antibrowning effect instantly after apple slices are dipped in its solution. Dipping apple slices in 1% ascorbic acid solution effectively inhibited browning (3). The rate of browning index was significantly reduced in peach slices with suppression of PPO activity (6). The combination treatment of 0.5% ascorbic acid, 0.01% 4-hexylresorcinol, and 1% calcium lactate extended shelf-life of fresh-cut pears from 15 to 30 days (7). To prolong the shelf-life of fresh-cut products, thermal processing has been traditionally used alone or in conjunction with chemical treatments. Thermal processing, however, tends to cause undesirable changes in food flavor, color, texture, and nutritional compounds. On the other hand, non-thermal processing has a minimal impact on the product quality and sensory properties (8,9).

Ultrasound has come into considerable notice in the food industry as its advance effects in food preservation (10). The use of ultrasound which utilizes the energy produced from sound waves leads to a bactericidal effect in microorganisms and enzyme inactivation by cell disruption during food processing because cavitation bubbles generate spots of extremely high pressure and temperature when they are imploded inside the cell (8,9). The enzyme destruction by the combination treatment of heat and ultrasonic waves considerably increases with ultrasonic wave amplitude and decimal reduction times at constant temperature decreased logarithmically as amplitudes increase (11). The decimal reduction time of peroxidase at 80°C reduces from 65 to 10 min when ultrasound is applied at 20 kHz (12). Generally, the resistance of enzymes to ultrasound is very high. In order to enhance the efficiency of ultrasound in food preservation, thus, it can be combined with other treatments such as chemicals (13). However, the application of ultrasounds on fresh-cut fruits and vegetables in order to inhibit browning has not been reported to our knowledge. The objective of this study was to investigate the effects of ultrasound with ascorbic acid on the quality of fresh-cut apples in order to prolong its fresh-like shelf-life.

Materials and Methods

Materials 'Fuji' apples that were harvested at the farm of Kyungpook National University in Republic of Korea on November 2008 were kept at 4°C, and processed within 2 days. Folin-Ciocalteu's reagent was purchased from Junsei Chemical Co. (Tokyo, Japan) and ascorbic acid,

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catechol, and chlorogenic acid were from Sigma–Aldrich (St. Louis, MO, USA). The 1% ascorbic acid solutions were cooled in a refrigerator before use.

Sample preparation The apples were sliced horizontally, manually cut into approximately 1.5 cm cubes free from core then peeled with a sharp stainless steel knife. After the preparation, 60 apple cubes were randomly selected and immediately applied with various treatments. The control sample (Cont) was dipped in distilled water while the other samples were treated as follows with ultrasound only (US), with 1% ascorbic acid only (AA), with 1% ascorbic acid and ultrasound (AS). Ultrasound was applied at a fixed frequency of 40 kHz. The remaining water in sample was drained using a vegetable spinner, 15 apple cubes were packed in polypropylene bags (10×10 cm, 0.04 mm thick) and sealed. The samples were stored at 4°C in the refrigerator and 3 bags were used for various analyses which were performed after 0 (the treatment day), 4, 8, and 12 days storage.

Color measurement Color of apple cubes was determined by means of a colorimeter (CR-200; Minolta Co., Osaka, Japan). The color were expressed as L (lightness), a (green to red), and b (blue to yellow) and ΔL, Δa, and Δb values were based on untreated apple cut surface in order to compare to browning degree of samples. A white ceramic plate was used for calibrating the instrument (L=97.78, a=–0.39, b=2.05). Total color change, ΔE was also computed as an indicator of the change in color as follows:

$$\Delta E=\sqrt{(L_t-L_{t_0})^2+(a_t-a_{t_0})^2+(b_t-b_{t_0})^2}$$

where t and t_0 correspond to any time during the experiments and the time before any treatment, respectively. The inhibition rate of browning is expressed as follows:

$$\text{Inhibition rate } (\%)=(\Delta E_{control}-\Delta E_{treatment})100/\Delta E_{control}$$

Polyphenol oxidase activity Apple cubes (10 g) were homogenized for 1 min in 50 mM sodium phosphate buffer (pH 7.0) and 50 g/L polyvinylpyrrolidone with a homogenizer in ice bath. The homogenate was filtered through 4 layers of cheese cloth and centrifuged at 16,000×g for 20 min at 4°C. The supernatant solution to be used for the measurement of PPO activity was filtered through a Whatman No. 42 filter paper. The reaction mixture included crude enzyme solution (0.2 mL) and 20 mM catechol solution (2.8 mL). The unit of enzyme activity was defined as the 0.001 change in absorbance at 420 nm between 60 and 120 sec of reaction. The enzyme extraction and assay of the activity were done at least in triplicate at each experiment day.

Catechol treatment Catechol application was based on a method proposed by Weller *et al.* (14). Apples with similar size were selected, washed, and horizontally cut into circular disks of approximately 1.5 cm in thickness, including the core. Apple disks were individually applied by ultrasound and ascorbic acid as noted above. To verify the location and existence of polyphenol oxidase, 1.5 mL of 50 mM catechol solution or same volume of distilled water were dropped and evenly spread over the cut surface of apple slices. Treated slices were exposed to air at ambient conditions (24°C, 30-40% RH) for 6 hr.

Total phenolic content Total phenolic content was determined by using modified Folin-Denis method (15). Apple cubes (10 g) were homogenized in a mixture of methanol and water (4:1 v/v) for 2 min and were extracted in shaking bath for 24 hr. After filtration, 4 mL of samples diluted using distilled water was mixed with Folin-Ciocalteu's reagent. After 5 min, 1 mL of saturated sodium carbonate solution was added and reaction mixture had been allowed to stand for 1 hr at room temperature before measuring the absorbance at 725 nm with a spectrophotometer (Optizen 2120UV; Mecasys, Daejeon, Korea). Total phenol content was expressed as mg chlorogenic acid equivalents/100 g fresh weight using a calibration curve with chlorogenic acid.

Statistical analysis The results were expressed as mean ±standard error (SE) of the mean which corresponds for the 3 analytical replicates. Analysis of variance (ANOVA) and Duncan's multiple range tests were performed using the SAS program version 9.1 for windows to determine the effect of various treatments such as ultrasound and ascorbic acid on the quality of fresh-cut apples. The level of significance was set at $p<0.05$.

Results and Discussion

Effect of ultrasound and ascorbic acid on color The color changes and browning inhibition rate of all samples during 12 days storage are shown in Table 1. The notable changes of color were observed with storage time. Generally, it is considered that the color of fresh-cut fruits and vegetables is very important because appearance and freshness of products provides a basis for judgment to consumers at the time of purchase (16). In decades, many studies have been reported about browning which was observed on the cut surface of fresh fruit (3,17,18). The L values showed a decreasing trend in all treatments, indicating a loss of lightness of cut surface, while ΔL and Δa values increased towards red hue during whole storage days. A decrease in L value and an increase in a value are interpreted as a browning on the cut surface of fruits and vegetables (19,20). The decrement of L value during whole storage period indicates browning occurred continuously. Much lighter color in AS-treated samples was maintained although a degree of browning in Cont and US-treated samples remarkably increased during storage and became commercially undesirable. The significantly highest L values were also maintained in the apple cubes treated with AS among all the treatments during storage. US-treated sample showed considerably high ΔL and Δa values thus US treatment appeared to aggravate browning, although it has slightly higher L value at treatment day. Color changes during storage of orange juice applied with ultrasound assisted thermal treatment indicated similar overall changes of a and b values, but significantly higher L values, which means lighter product (21). In AA-treated samples, significantly higher L value thus lighter color was maintained after 8 days. However, after 12 days, L value of

Table 1. Color changes and browning inhibition rate of fresh-cut apple cubes treated with ultrasound and ascorbic acid during storage at 4°C for 12 days

Hunter's parameter	Day at 4°C	Sample[1)]			
		Cont	US	AA	AS
L	0	70.71±3.42$^{bA2)}$	71.71±2.88^{bA}	71.77±2.21^{bA}	73.46±1.87^{aA}
	4	70.66±3.14^{bA}	70.84±2.95^{bBA}	70.85±3.49^{bBA}	73.04±1.43^{aA}
	8	69.22±3.62^{cA}	69.53±2.11^{cB}	71.17±2.49^{bA}	73.05±3.09^{aA}
	12	69.01±4.25^{bA}	69.60±2.78^{bB}	69.27±3.28^{bB}	73.01±1.93^{aA}
a	0	-4.24±0.57^{aC}	-4.34±0.57^{aC}	-4.86±0.59^{bC}	-5.20±0.38^{cC}
	4	-3.98±0.32^{aBC}	-3.91±0.53^{aBC}	-4.20±0.38^{bB}	-5.23±0.32^{cC}
	8	-3.53±0.56^{bA}	-3.16±0.47^{aA}	-3.78±0.49^{bA}	-4.69±0.43^{cB}
	12	-3.78±0.95^{baBA}	-3.72±0.38^{aB}	-3.78±0.41^{baA}	-4.11±0.33^{bA}
b	0	23.30±2.63^{baB}	23.68±2.59^{aA}	21.85±2.43^{bBA}	19.49±2.78^{cA}
	4	23.96±2.80^{aB}	22.94±2.48^{aA}	21.27±2.64^{bB}	18.37±1.56^{cBA}
	8	23.30±2.28^{aB}	23.21±1.94^{aA}	22.76±2.31^{aA}	17.28±2.39^{bB}
	12	25.74±2.67^{aA}	22.73±2.87^{bA}	21.49±2.35^{bBA}	18.50±1.72^{cBA}
ΔL	0	5.73±3.42^{aA}	4.73±2.88^{aB}	4.67±2.21^{aB}	2.98±1.87^{bA}
	4	5.78±2.21^{aA}	5.60±1.87^{aBA}	5.59±3.14^{aBA}	3.40±2.95^{bA}
	8	7.22±3.62^{aA}	6.91±2.11^{aA}	5.27±2.49^{bB}	3.39±3.09^{cA}
	12	7.43±4.25^{aA}	6.84±2.78^{aA}	7.17±3.28^{aA}	3.43±1.93^{bA}
Δa	0	1.72±0.57^{aC}	1.62±0.57^{aC}	1.10±0.59^{bC}	0.76±0.38^{aC}
	4	1.98±0.32^{aBC}	2.05±0.53^{aBC}	1.76±0.38^{bB}	0.73±0.32^{cC}
	8	2.43±0.56^{bA}	2.80±0.47^{aA}	2.18±0.49^{bA}	1.27±0.43^{cB}
	12	2.18±0.95^{baBA}	2.24±0.38^{aB}	2.18±0.41^{baA}	1.85±0.33^{bA}
Δb	0	3.81±2.63^{baB}	4.19±2.59^{aA}	2.36±2.43^{bBA}	0.00±2.78^{cA}
	4	4.47±2.80^{aB}	3.45±2.48^{aA}	1.78±2.64^{bB}	1.12±1.56^{cBA}
	8	3.81±2.28^{aB}	3.72±1.94^{aA}	3.27±2.31^{aA}	2.21±2.39^{bB}
	12	6.25±2.67^{aA}	3.24±2.87^{bA}	2.00±2.35^{bBA}	0.99±1.72^{cBA}
ΔE	0	7.47±3.63^{aB}	6.85±3.28^{aA}	5.73±2.60^{aB}	4.00±2.13^{bB}
	4	7.95±3.43^{aB}	7.17±3.29^{aA}	6.64±3.52^{aBA}	4.03±1.25^{bB}
	8	8.89±3.44^{aBA}	8.55±2.15^{aA}	6.88±2.75^{bBA}	5.29±2.25^{cA}
	12	10.39±4.10^{aA}	8.45±2.59^{bA}	8.14±3.16^{bA}	4.57±1.37^{cBA}
Browning inhibition rate (%)	0	-	8.4	23.4	46.5
	4	-	9.7	16.4	49.3
	8	-	3.8	22.6	40.5
	12	-	18.7	21.6	56.0

[1)]Cont, control; US, treated with ultrasound at 40 kHz in distilled water; AA, just dipped in 1% ascorbic acid; and AS, treated with ultrasound in 1% ascorbic acid solution.
[2)]Each value is expressed as the mean±SD (n=25); Means followed by different letters within the row (a-d) and within the column (A-D) are significantly different (p<0.05).

AA-treated samples had similar level to Cont and US-treated samples and secondary browning was noticeable in AA-treated samples. Ascorbic acid reduces quinones which produced by PPO-catalyzed oxidation of polyphenols and back to dihydroxy polyphenols. However, when the reducing power of ascorbic acid is depleted quinones will accumulate, and browning rapidly occurs (3). The AS-treated sample maintained light color throughout the whole storage period by sensory evaluation (data not shown). The lowest ΔL value and the smallest change of Δa and Δb values indicate little color change occurred in AS.

AS-treated samples maintained significantly high levels of ΔE values until 4 days (Table 1). Cont and US-treated samples showed the highest ΔE values over all the storage period. The increase of ΔE in Cont and US-treated samples was noticeable with significantly lower lightness and higher redness and yellowness during storage. Cont and AS-treated samples had the significantly highest and lowest ΔE at 12 days, respectively.

The browning inhibition rate based on ΔE was shown to be higher in AS-treated samples as compared to other samples. AS-treated samples had more than 40% inhibition rate during the storage days. This result showed that AS treatment is effective on inhibiting the browning of fresh-cut apples.

Effect of ultrasound and ascorbic acid on PPO activity

The use of ultrasound in conjunction with ascorbic acid was found to influence on the PPO activity during storage (Fig. 1). All samples except for AS-treated samples showed

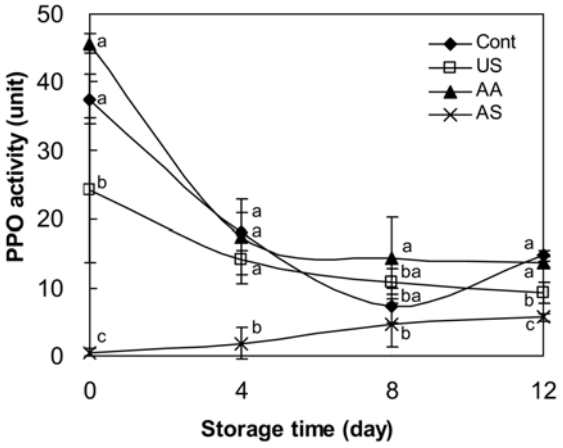

Fig. 1. PPO activity of fresh-cut apple cubes treated with ultrasound and ascorbic acid during storage at 4°C for 12 days. Treatments with significant difference from one another are expressed as the different letter by Duncan's multiple range tests ($p<0.05$). Cont, control; US, treated with ultrasound at 40 kHz in distilled water; AA, just dipped in 1% ascorbic acid; and AS, treated with ultrasound in 1% ascorbic acid solution.

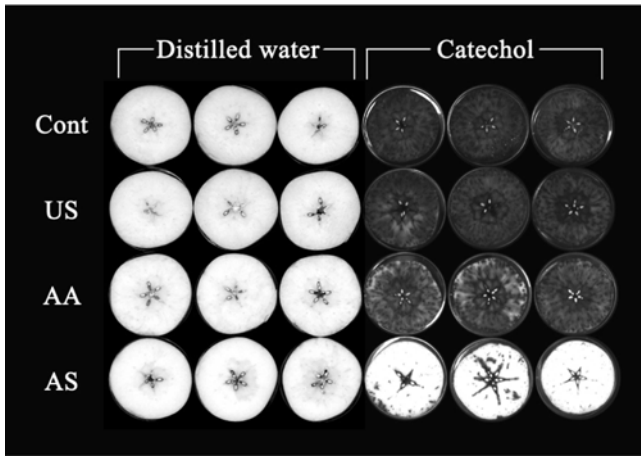

Fig. 2. Polyphenol oxidase reaction with catechol on apple disks treated with ultrasound and ascorbic acid. Cont, control; US, treated with ultrasound at 40 kHz in distilled water; AA, just dipped in 1% ascorbic acid; and AS, treated with ultrasound in 1% ascorbic acid solution.

a decreasing trend and PPO activity decreased for 8 days of storage and increased thereafter in Cont. US and AS-treated samples showed lower PPO activities than other samples. Moreover, AS-treated samples had much lower PPO activity than AA-treated samples. Arias *et al.* (22) reported that PPO activity in the existence of increasing concentrations of ascorbic acid was constantly maintained, however, the lag phase considerably increased with the ascorbic acid concentration. Therefore, our results also imply that ascorbic acid mainly acts as an enzymatic browning inhibitor, not as a direct inhibitor of PPO and combined treatment of ultrasound and ascorbic acid has different inhibition mechanism on PPO activity from ascorbic acid treatment only. Lower level in PPO activity of AS-treated sample was observed as compared to samples treated with either ultrasound or ascorbic acid during the whole storage period, ranging from 0.50±0.50 to 5.67±0.58. It was reported that there was a direct correlation between PPO activity and degree of browning for various cultivars of apple (23). Our results indicate that the reduction of PPO activity has influence on the antibrowning effect in AS.

It was known that ultrasound has an effect on enzyme inactivation by cell lysis and severe shear stress, which were caused by the energy produced by great vibrations and acoustic streams within the adjacent liquid to bubble. It was also revealed that the combined application of heat and ultrasonic waves can be more effectively contribute to the enzyme inactivation (8). Manothermosonication have been studied in the inactivation of various enzymes such as peroxidase, lipoxigenase, PPO, or pectinmethylesterase, which make food quality deteriorate. On enzyme denaturation, a synergistic effect of heat and ultrasonic waves was observed in watercress and enzyme solution applied with different temperatures and frequencies, respectively (11, 24). The pectinmethylesterase activity of fresh lemon juice was effectively decreased when ultrasound assisted thermal treatment was applied and more than 90% inactivation was shown at 80°C with ultrasonication (21). It was also reported that the resistance of enzymes to ultrasound is very high and it may need ultrasonication treatment for many long hours to achieve the desired result and ultrasound alone as a preservation technology is insufficient with respect to enzyme inactivation (12). Our results showed the potentiality of PPO inactivation from the application of ultrasound in combination with ascorbic acid without heat treatment distinctively from several established studies. Antibrowning effect of AS treatment was also showed to be correlated with low PPO activity.

Effect of ultrasound and ascorbic acid on the distribution of PPO Color change which can be seen in different parts of the cut surface of sliced apples after each treatment was observed as shown in Fig. 2. PPO was not uniformly distributed in the cross section of apple, being predominant at the core and secondarily near the skin by immunostaining of the membrane (25). The intense color change about the core was also observed after the catechol was applied in this study. The pigments developed when catechol was oxidized by the action of PPO. For the fact that the lack of browning was observed in the rest of the tissue, there are 2 possible explanations. One is that the enzyme was present in a lower concentration and the other is that it was in a latent form with little reactivity toward catechol (14). These support the idea that the distribution of the PPO can be explained in terms of the localization of brown pigment. The evidence that AS treatment has a synergistic effect on PPO activity can be seen in Fig. 2. AS-treated samples showed much lighter color with exception of scattered browning around core, in contrast to Cont and US-treated samples which had severe change in color all over samples. AS treatment also showed a noteworthy effect on inhibition of browning at the core. On the other hand, much wider distribution of brown pigment was observed on AA-treated samples, contrary to what happens on AS-treated samples. This phenomenon also proves that it is not that ascorbic acid directly inhibits PPO

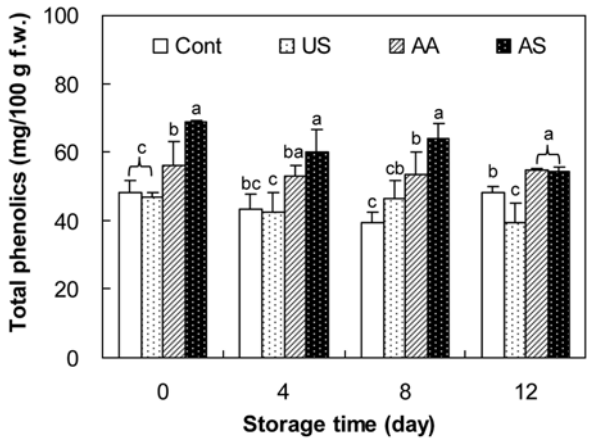

Fig. 3. Total phenolic contents of fresh-cut apple cubes treated with ultrasound and ascorbic acid during storage at 4°C for 12 days. Treatments with significant difference from one another are expressed as the different letter on the bar graph by Duncan's multiple range tests ($p<0.05$). Cont, control; US, treated with ultrasound at 40 kHz in distilled water; AA, just dipped in 1% ascorbic acid; and AS, treated with ultrasound in 1% ascorbic acid solution.

activity but delays browning as an antioxidant compound (22). Consequently, there is considerable validity in this theory that ultrasound application with ascorbic acid effectively reduced browning caused by PPO activity of fresh-cut apples.

Effect of ultrasound and ascorbic acid on total phenolic content Apple contains several phenolic compounds which has antioxidant activity and bitter taste (26). Discoloration of apple flesh is mainly due to enzymatic oxidation of phenolic compounds consumed as substrates of PPO. Total phenolic contents of apple samples with different treatments are illustrated in Fig. 3. There was no significant trend in the total phenolic content of treated apple cubes during storage days. The US-treated samples showed the lowest content but not significantly different with Cont. Ultrasound-assisted extraction at 20 kHz (100 W) which makes analytes possible to extract from certain substances in shorter time with simultaneous hydrolysis of various phenolic compounds, was used in order to determine phenolic compounds in strawberries (27). Accordingly, it is assumed that phenolic compounds leaked out from samples by ultrasound treatment. On the other hand, total phenolic contents were higher at the apple slices dipped in a solution of 1% ascorbic acid and 1% citric acid for 3 min as compared to those control samples for all the storage period, because of the reducing action of ascorbic acid that our results showed significantly higher contents of total phenolics in AA and AS-treated samples during the whole storage period and were in accordance with the findings of the above-mentioned study. Especially, the significantly highest level in total phenolic contents of AS-treated samples was observed. Several studies have focused on phenolic compounds induced by abiotic stress. Dixon and Paiva (28) reported that many phenolic compounds were induced in response to wounding. Ke and Saltveit (29) also noted that wounding stimulates producing more phenolics in order to initiate repair processes at cells adjacent to the injury. In general, when ultrasound propagates into a liquid, high acoustic pressure makes modification of the cellular activity and puncturing of the cell wall (12). Total phenolic contents in US-treated samples slightly fluctuated with similar trends to AS. These indicate that higher phenolic contents observed in apple cubes treated by AS is considered to be attributable to a simultaneous effect of both ascorbic acid as an antioxidant and wound-induced phenolic accumulation produced by ultrasound. A decreasing trend in total phenolic contents with a slight increment in PPO activity was also observed in AS treatment samples during whole storage period. These results show that AS treatment inactivate PPO, thus, phenolic compounds were not consumed as enzyme substrate. We can therefore conclude that AS treatment is very effective to inhibit PPO activity thus can be use for apple antibrowning. Moreover, it is worth noting that AS-treated samples displayed high level of phenolic compounds which is beneficial because of the consumers increased interest in healthy foods.

Ultrasound treatment with antibrowning agent effectively inhibited the browning by inhibiting the PPO activity and effectively maintained the high amount of phenolic compounds of fresh-cut apples. Our study revealed that the use of ultrasound in combination with ascorbic acid had a positive effect on quality of fresh-cut apples and suggested to be a promising processing technology for fresh-cut fruits and vegetables.

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