



Effect of Combined Ultrasonication and Modified Atmosphere Packaging on Storage Quality of Pakchoi (*Brassica chinensis* L.)

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Abstract

Feasible use of ultrasonic treatment (UT) in combination with modified atmosphere packaging (MAP) to preserve pakchoi was evaluated. Pakchoi was treated with ultrasound (30 kHz) for different periods of time (5 min, 10 min, and 15 min) prior to MAP (with 5% O₂ + 10% CO₂ + 85% N₂); the treated vegetables were then stored at 4 °C for 30 days. UT in combination with MAP could retard an increase in the total number of colonies in pakchoi during the storage. UT-10 min decreased the initial total number of colonies in pakchoi from 7.11 to 6.01 log CFU/g and effectively slowed down the increase in mass loss, yellowing, cell membrane permeability, and malondialdehyde content of the stored vegetable; such a treatment also slowed down the decrease in total soluble solids and losses of ascorbic acid and chlorophylls. The synergistic effect of UT and MAP results in effective reduction of peroxidase and polyphenol oxidase activities. In conclusion, UT-10 min + MAP was the best method to preserve pakchoi for 30 days.

Keywords Cellular damage · Enzyme activity · Modified atmosphere packaging · Pakchoi · Storage quality · Ultrasound treatment

Introduction

Pakchoi (*Brassica chinensis* L.) is a Chinese cabbage and is one of the major edible leafy vegetables of China (Du et al. 2008). Pakchoi leaves are bright green in color and are sweet and rich in various vitamins and minerals. The vegetable,

however, suffers water loss, wilting, and yellowing during storage and shipping; the fresh quality of pakchoi is then lost, and its shelf life is shortened. A method to extend the freshness and preserve the storage quality of pakchoi is therefore highly desirable.

Refrigerated storage is one of the most commonly employed methods to delay deterioration of fresh fruits and vegetables since such a practice helps inhibit biochemical reactions as well as microbial activity (Alexandre et al. 2012). More recently, modified atmosphere packaging (MAP) has also noted to exhibit capability to limit deleterious oxidation reactions and alter the respiration rate as well as reduce water loss from a produce (Caleb et al. 2013). MAP has been recognized as an efficient method to extend the shelf life as well as preserve the quality of a variety of fresh products (Alak et al. 2011). MAP with 2–5% oxygen was noted to help reduce the respiration rate and senescence of fruits and vegetables (Jayas and Jeyamkondan 2002). Goliáš et al. (2012) reported that low-concentration O₂ and high-concentration CO₂ atmosphere could effectively help retain the flavor of sweet cherry; up to 80% of the total volatile compounds was maintained after the storage period. Teixeira et al. (2016) also reported that low-O₂ and high-CO₂ environment could prolong the shelf life of guava. Unfortunately, prior study on MAP of pakchoi is extremely limited, if not unavailable. For this

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reason, we had adopted the gas composition that has been found effective for other produce as a starting point in our work. More importantly, our focus here is on the feasible use of combined ultrasonication and MAP to improve the storage quality of pakchoi.

In an attempt to improve the capability of MAP to prolong the shelf life and preserve the quality of fresh produce even further, additional appropriate processing technology should be used in combination with MAP. Among the possible technologies, ultrasound is one of the most promising. Ultrasound is classified as a non-thermal technology that creates cavitation and mechanical effects that can be used to enhance the preservation effects of other technologies for foods (Mason et al. 1996). Various ultrasound-related technologies have indeed been applied to assist the processing and preservation of fruits and vegetables (Mothibe et al. 2011). Ultrasonic treatment was, for example, applied to preserve juices and maintain nutrition and quality of fruits and vegetables (Paramjeet and Gogate 2016). Cassani et al. (2017) reported that the combined use of ultrasound and vanillin was effective in improving the taste, quality, and safety of strawberry juice; the juice exhibited reduced microbial development and possessed acceptable sensory quality. Ultrasound requires lower energy consumption and is inexpensive. It is also environmentally friendly and highly safe (Chemat et al. 2011).

Mansur and Oh (2015) evaluated the efficacy of thermosonication (TS) in combination with slightly acidic electrolyzed water (SAcEW) to extend the shelf life of fresh-cut kale during the refrigerated storage. The vegetable was inoculated with *Listeria monocytogenes* and treated with TS + SAcEW. TS + SAcEW was noted to be effective in prolonging the shelf life of kale, with an extension of around 4 days and 6 days at 4 °C and 7 °C, respectively. Millan-Sango et al. (2015) also noted the ability of ultrasound, when used in combination with essential oils, to decontaminate *Salmonella* inoculated on lettuce leaves. Char et al. (2010) studied the combined use of ultrasound and UV-C light to inactivate microorganisms in fruit juices. UV-C light was noted to exhibit limited capability to inactivate microorganisms in clear and non-opaque juice media. The combined use of UV-C light and ultrasound was nevertheless noted to be effective in inactivating the microorganisms in orange juice, which is non-clear and opaque.

Although there already are studies on the use of ultrasound to extend the shelf life of various fruits and vegetables, most investigations focused only on microbial inactivation; limited focus was made on the effect of the treatment on the evolutions of key quality characteristics and enzyme activities, which may play an important role on quality deterioration during storage. Among the limited available works, Feng et al. (2018) conducted a study on cucumber postharvest storage quality. These investigators applied ultrasonic treatment prior to controlled atmosphere packaging to

preserve the flavor-related volatiles and storage quality of cucumber, helping the produce to be preserved longer. However, the use of ultrasound as a pretreatment of fruits and vegetables prior to storage is still only at a small-scale experimental phase (Xu et al. 2013).

Based on the aforementioned lack of information, this study was conducted to evaluate the effect of ultrasonic treatment in combination with MAP on the quality of pakchoi during storage at 4 °C ± 1 °C and 90% ± 4% relative humidity. The total number of colonies, mass loss, color, cell membrane permeability, malondialdehyde content, total soluble solid content, ascorbic acid content, and chlorophyll content as well as activities of peroxidase and polyphenol oxidase of pakchoi were evaluated.

Materials and Methods

Chemicals and Reagents

The following chemicals and reagents were used: plate count agar, sterile saline, trichloroacetic acid, 2-thiobarbituric acid, acetic acid-sodium acetate buffer, guaiacol solution, hydrogen peroxide solution, catechol solution, oxalic acid, and 80% (v/v) acetone solution. Plate count agar, acetic acid-sodium acetate buffer, guaiacol solution, hydrogen peroxide solution, catechol solution, oxalic acid, and acetone were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China); trichloroacetic acid and 2-thiobarbituric acid were obtained from XYZ (Darmstadt, Germany).

Preparation of Test Materials

Fresh pakchoi was handpicked from a local vegetable farm in Wuxi, Jiangsu, China. After harvesting, pakchoi was immediately transported to the lab and sorted. Only the vegetable with normal color, no yellowing, browning, and mechanical damage was used for the experiments.

Ultrasonic Treatment

Fresh pakchoi was immersed in an ultrasonic bath (JY98-3D; Ningbo Ultrasonic Instrument Co., Zhejiang, China) at a fixed frequency of 30 kHz and a power intensity of 2.4 W/g for either 5 min, 10 min, or 15 min; the vegetable was then drained. The volume of the bath was 2 L, with deionized water as the medium; the temperature of the medium was controlled to be 20 °C ± 1 °C throughout the whole treatment process. The control vegetable sample was soaked in deionized water without sonication for 5 min and then drained. Excess water on the vegetable surface was carefully removed with paper towel (Knorr et al. 2004).

Packaging and Storage of Test Materials

Pakchoi ($200 \text{ g} \pm 2 \text{ g}$) was placed in a 48- μm -thick high-density polyethylene bag ($40 \text{ cm} \times 35 \text{ cm}$) (Zhang et al. 2006). The bag has an oxygen permeability of $4.5 \text{ mL/m}^2/\text{day/atm}$ at $25 \text{ }^\circ\text{C}$, with 100% relative humidity (RH), and a water vapor transmission rate of $8 \text{ g/m}^2/\text{day}$ at $40 \text{ }^\circ\text{C}$, with 90% RH (Zhang et al. 2006; An et al. 2007). Gas flushing was conducted in a vacuum-emptied package using a gas mixer (K. Kang KK-180, Suzhou, China). The gas replacement type of MAP was vacuum filling replacement (Mannheim and Nehama 2010; Lagnika et al. 2013). The initial in-package gas concentration was determined to be 5% O_2 and 10% CO_2 ; the content was balanced with N_2 (Izumi and Inoue 2017; Goliáš et al. 2012). The control sample was stored without being packed. All the samples were stored at $4 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ and $90\% \pm 4\%$ RH for 30 days. The samples were taken and analyzed for storage quality at every 5 days (0 day, 5 days, 10 days, 15 days, 20 days, 25 days, and 30 days). The samples were labeled as MAP (no ultrasonic treatment), UT-5 min + MAP (ultrasonic treatment for 5 min), UT-10 min + MAP (ultrasonic treatment for 10 min), and UT-15 min + MAP (ultrasonic treatment for 15 min).

Determination of Quality Parameters

Total Number of Colonies

Referring to the GB 4789.2-2016 standard, the 10-g sample was mixed with 90 mL of sterile saline to prepare a sample homogenate. The content was diluted sequentially to achieve homogenates of three dilutions, namely 10^{-4} , 10^{-5} , and 10^{-6} ; 1 mL of each homogenate was added to a sterile culture dish. One milliliter of sterile saline was used as a blank control. The plate was added with plate count agar medium and rotated to mix the content. After $48 \text{ h} \pm 2 \text{ h}$ of incubation at $36 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$, the total number of colonies was counted. The data were recorded as colony-forming units (CFU) and are expressed as $\log \text{ CFU/g}$.

Mass Loss

Mass loss was calculated as the percentage change of the initial mass. Weighing was carried out with package to avoid moisture condensation on a package (Manolopoulou et al. 2012). All measurements were performed in triplicate, and the average values are reported.

Color

Color was measured in CIE $L^*a^*b^*$ chromatic space with a chroma meter (CR-400; Minolta, Osaka, Japan) as per the suggested methods of Feng et al. (2018) and Huang et al.

(2014). The meter was calibrated against the manufacturer's standard white ceramic tile. L^* indicates the lightness/darkness of a sample, a^* indicates the redness/greenness, and b^* measures the yellowness/blueness of a sample. Three portions of pakchoi were selected from each test group; each sample was measured 9 times.

Total Soluble Solid Content

Soluble substances (mainly soluble sugars) in fruits and vegetables can directly reflect the maturity and quality of a sample. In this study, total soluble solid (TSS) content in terms of $^\circ\text{Brix}$ of a sample was determined by a handheld refractometer (Aipli, Hangzhou, China). Five grams of pakchoi leaves was blended to obtain juice for the measurement (Feng et al. 2018). All measurements were performed in triplicate, and the average values are reported.

Cell Membrane Permeability

Electrical conductivity of a sample exudate was first measured as per the suggested methods of Wang et al. (2015) with some modification. Twenty slices were randomly obtained from pakchoi leaves via the use of a 6-mm-diameter-hole puncher. The slices were placed in a 50-mL beaker with 20 mL deionized water; the content was moderately shaken to remove surface dirt. The beaker was then covered with gauze, and the content was poured out to remove the water and dirt. Thirty milliliters of deionized water was again added to the beaker; the content was left to stand for 1 h. The conductivity (W_1) was then measured using a conductivity meter (3100C; Starter, Shanghai, China). Conductivity (W_2) was measured again after placing the film-wrapped beaker and its content in boiling water for 15 min. The relative conductivity (W_0) was calculated as $W_1 / W_2 \times 100 (\%)$. All measurements were performed in triplicate, and the average values are reported.

Malondialdehyde Content

Malondialdehyde (MDA) content, which is an indirect indicator of lipid oxidation-induced cellular damage, was determined using thiobarbituric acid (TBA) assay (Meng et al. 2012; Chen et al. 2017). One gram of a sample was mixed with 5 mL of 100 g/L trichloroacetic acid and then centrifuged at $10,000 \times g$ at $4 \text{ }^\circ\text{C}$ for 20 min. Two milliliters of the supernatant was added with an equal volume of 6.7 g/L 2-thiobarbituric acid. The mixture was put into boiling water for 20 min and centrifuged once more after being taken out and cooled. Absorbance was then measured at 450 nm, 532 nm, and 600 nm. MDA content was then calculated as per Eq. (1)

MDA content ($\mu\text{mol}/\text{kg}$)

$$= (6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}) \times V_1 \times V_2 / (V_3 \times W) \quad (1)$$

where A_{600} , A_{532} , and A_{450} are the absorbance values at 600 nm, 532 nm, and 450 nm, respectively; V_1 is the total volume of the solution obtained after the reaction (mL); V_2 is the total volume of the extract solution (mL); V_3 is the volume of the extract solution used for the reaction (mL); and W is the mass of the sample (g). All measurements were performed in triplicate, and the average values are reported.

Ascorbic Acid Content

Ascorbic acid content was determined using the 2,6-dichlorophenolindophenol assay (Alexandre et al. 2011; Darvishi et al. 2019) and is reported as milligrams per 100 g of the initial sample mass. Measurements were made at the beginning and during the storage. A sample of $10 \text{ g} \pm 0.5 \text{ g}$ was first weighed with an appropriate amount of 20 g/L oxalic acid solution. A small amount of quartz sand was added; the content was ground into a homogenate in an ice bath. The homogenate was dissolved in 20 g/L oxalic acid solution and diluted with 20 g/L oxalic acid solution to 100 mL; the content was shaken and filtered after being left to stand for 10 min. Ten milliliters of the filtrate was titrated with 2,6-dichlorophenolindophenol. The end-point of the titration was when the solution became red. Titration with 20 g/L oxalic acid solution was noted as blank control. All measurements were done in triplicate, and the average values are reported. The ascorbic acid content was calculated as per Eq. (2)

Ascorbic acid content ($\text{mg}/100 \text{ g}$)

$$= ((V - V_0) \times c \times V_1 \times A \times 100) / M(V_2 - V_0) \quad (2)$$

where V , V_0 , and V_2 are the volumes of the 2,6-dichloroindolol solution consumed by titration of the sample, blank control, and ascorbic acid standard solution (mL); c is the concentration of the ascorbic acid solution (mg/mL); V_1 is the volume of the ascorbic acid standard solution; A is the dilution factor (10); and M is the mass of the sample (g).

Chlorophyll Content

Chlorophyll content of a sample was measured as per the suggested methods of Meng et al. (2012) with some modification. A sample of $0.20 \text{ g} \pm 0.01 \text{ g}$ with leaf vein removed was mixed with an appropriate amount of calcium carbonate and quartz sand. A small amount of 80% (v/v) acetone solution was added to the mixture; the whole content was ground into a homogenate. Acetone solution was continuously added, and grinding continued until the vegetable tissue became

white. The content was left to stand for 3–5 min, filtered, washed, and again filtered. The second filtrate was transferred into a brown volumetric flask and diluted with 80% (v/v) acetone solution to 25 mL. The absorbance value was measured at 645 nm and 663 nm. All measurements were performed in triplicate, and the average values are reported. The chlorophyll content was calculated as per Eq. (3)

Chlorophyll content (mg/g)

$$= (20.29 \times A_{645} + 8.05 \times A_{663}) \times V / M \times 1000 \quad (3)$$

where A_{645} and A_{663} are the absorbance values at 645 nm and 663 nm, respectively; V is the total volume of the solution obtained after the reaction (mL); and M is the mass of the sample (g).

Peroxidase Activity

Peroxidase (POD) activity was determined as per the recommended methods of Meng et al. (2012) with some modification using a spectrophotometer (UV-visible 2600; Precision Science Instrument, Shanghai, China). The absorbance of the reaction system was measured at 470 nm (ΔOD_{470}); the evolution curve of the OD value was then plotted. The change of the absorbance per minute was calculated based on the initial linear part of the curve. A peroxidase activity unit ($\Delta\text{OD}_{470}/(\text{min}/\text{g})$) was noted as the change of the absorbance value per minute per gram (fresh mass) of the sample. All measurements were performed in triplicate, and the average values are reported.

Polyphenol Oxidase Activity

Polyphenol oxidase (PPO) activity was determined as per the recommended methods of Meng et al. (2012) with some modification. PPO activity was assayed using a spectrophotometer (UV-visible 2600; Precision Science Instrument, Shanghai, China) at 420 nm; the evolution curve of the OD value was then plotted. Calculation of the change of the absorbance value per min was based on the initial linear part of the curve and is denoted as ΔOD_{420} . A polyphenol oxidase activity unit ($\Delta\text{OD}_{420}/(\text{min}/\text{g})$) is expressed as the change of the absorbance value per minute per gram (fresh mass) of the sample. All measurements were performed in triplicate, and the average values are reported.

Statistical Analysis

Statistical analysis of the experimental data was performed using SPSS software for Windows (version 11.5.1; SPSS Inc., Chicago, IL). All the data were subject to one-way analysis of variance (ANOVA). Differences among the product

quality attributes were tested at 95% confidence level via the use of Duncan’s new multiple range tests.

Results and Discussion

Total Number of Colonies in Pakchoi During Storage

The total number of colonies in pakchoi which undergone different treatments during storage is shown in Fig. 1. The initial total number of colonies in the control as well as in the samples subjected to MAP, UT-5 min + MAP, UT-10 min + MAP, and UT-15 min + MAP was 7.11 log CFU/g, 7.11 log CFU/g, 6.48 log CFU/g, 6.01 log CFU/g, and 6.41 log CFU/g, respectively. The microbial loads belonging to ultrasound-treated samples were lower than those of the control but were not significant ($P > 0.05$). The total number of colonies increased at prolonged storage time. After 15 days of storage, the total number of colonies rapidly increased. At the end of the storage, the total number of colonies in the control and samples subjected to MAP, UT-5 min + MAP, UT-10 min + MAP, and UT-15 min + MAP was 8.56 log₁₀ CFU/g, 8.41 log₁₀ CFU/g, 8.36 log₁₀ CFU/g, 8.13 log₁₀ CFU/g, and 8.38 log₁₀ CFU/g, respectively.

Although the application of the combined treatment did not show much improvement in terms of microbial load reduction, such a treatment was noted to be effective in maintaining the other quality characteristics of pakchoi as will be discussed in the next sections.

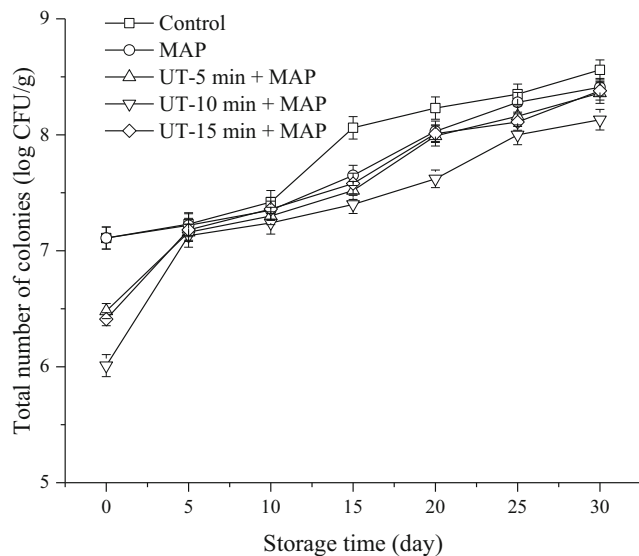


Fig. 1 Change of the total number of colonies of pakchoi during the refrigerated storage. Values are the means of three replicates, and bars stand for standard deviations

Mass Loss of Pakchoi During Storage

Mass loss of fruits and vegetables reflects the water loss caused by respiration (Jiang 2013; Xin et al. 2013). Mass losses of pakchoi which undergone different treatments during storage are shown in Fig. 2. During the storage period of 30 days, mass losses of all experimental samples gradually increased. Significant differences in the mass loss were observed between the control and UT + MAP groups ($P < 0.05$). The mass loss of UT-10 min + MAP was the lowest at 1.878% ± 0.062%; this value was lower than the acceptable limit of 2% as mentioned by Manolopoulou et al. (2010). This implies that ultrasonic treatment for 10 min in combination with MAP could effectively delay the increase in mass loss. This could be due to the preservation of hydrogen bonding between water molecules and macromolecules in pakchoi upon ultrasonication, which led, in turn, to reduced water loss (Feng et al. 2018). Fan et al. (2019) similarly reported that ultrasound treatment for 10 min could effectively reduce the mass loss of modified atmospheric packaged fresh-cut cucumber.

After storage for 20 days, the mass loss of UT-15 min + MAP appeared to be accelerated compared with those belonging to other UT + MAP groups. This may be due to the destructive effect of ultrasound at extended application time on the leaf surface membrane, which, in turn, caused more water loss. Although ultrasonic treatment in combination with MAP appears to be a promising means for preservation of leafy vegetables, extended ultrasonic treatment time should not be used.

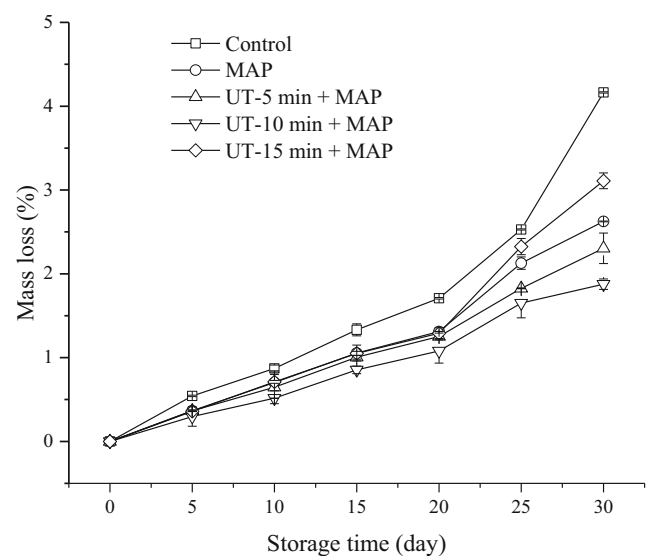


Fig. 2 Change of the mass loss of pakchoi during the refrigerated storage. Values are the means of three replicates, and bars stand for standard deviations

Color of Pakchoi During Storage

Color reflects the edible value of vegetables (Zhang et al. 2003). Here, the L^* value represents the lightness of pakchoi, while the b^* value represents the degree of yellowing of pakchoi. The results are shown in Table 1. In all cases, L^* exhibited a decreasing trend. Throughout the storage period, the L^* value of the control was significantly ($P < 0.05$) lower than that of the treated samples. As can be seen in Table 1, the b^* value showed an increasing trend upon the storage; the higher b^* value indicates the yellower leaves. This is because pakchoi was noted to wilt and became yellower at the prolonged storage period. The b^* values of UT + MAP samples were significantly ($P < 0.05$) lower than those of the control. The treated samples showed unobvious yellowing throughout the whole storage period. UT-10 min + MAP was noted to result in the sample with the lowest b^* value. This could be due to the appropriate cavitation effect, which helped decrease the metabolic rate of pakchoi, resulting, in turn, in the lowest yellowing and highest lightness (Feng et al. 2018). The observation here is consistent with that of Birmpa et al. (2013) that ultrasonic treatment (at 30 W/L) for 10 min could effectively preserve the color of fresh-cut lettuce and strawberry.

Cell Membrane Permeability of Pakchoi During Storage

Cell membrane permeability is expressed through the leakage of ions from plant tissues (Duan et al. 2009). When cell membranes of fruits and vegetables are damaged, electrolyte leakage causes an increase in the electrical conductivity of an exudate.

Measuring the exudate conductivity could therefore give information on cell membrane permeability (Chen et al. 2017).

Changes of cell membrane permeability of pakchoi which undergone different treatments during storage are shown in Fig. 3. The permeability gradually increased with the increased storage time in all cases, implying the deterioration of cell membrane; the higher the permeability, the less integrity of the cell membrane (Chen et al. 2017). The cell membrane permeability of pakchoi was $8.35\% \pm 0.13\%$ at the beginning of the storage and increased to $22.98\% \pm 1.85\%$ in the case of UT-10 min + MAP and $45.23\% \pm 11.05\%$ in the case of the control at the end of the storage. The cell membrane permeability of the sample subjected to UT-10 min + MAP indeed exhibited the slowest increase throughout the whole storage period; such a rate was significantly lower from that of the control ($P < 0.05$).

The increased cell membrane permeability is related to the increased activities of cell wall-degrading enzymes (Brummell and Harpster 2001; Tessmer et al. 2016). The cavitation effect of ultrasound could inhibit the activities of those enzymes and thus lowered the rate of cell membrane damage. Excessive application of ultrasound, however, could damage the cell membrane as mentioned in the earlier section on mass loss.

MDA Content of Pakchoi During Storage

MDA is a product of membrane lipid peroxidation, and its content is positively correlated with the degree of cell membrane damage (Xing et al. 2010). MDA can react with proteins

Table 1 Color change of pakchoi during the refrigerated storage

Color	Experimental group	Storage period (days)					
		0	5	10	15	25	30
L^*	Control	57.99 ± 1.97 ^a	49.66 ± 2.36 ^b	47.88 ± 2.99 ^b	47.36 ± 4.73 ^b	54.81 ± 3.08 ^a	38.64 ± 2.95 ^c
	MAP	57.99 ± 1.97 ^a	51.15 ± 2.46 ^{ab}	51.42 ± 2.19 ^a	51.92 ± 2.28 ^a	56.08 ± 2.82 ^a	46.86 ± 3.83 ^b
	UT-5 min + MAP	57.99 ± 1.97 ^a	49.80 ± 1.02 ^b	48.65 ± 0.81 ^b	54.56 ± 2.50 ^a	56.73 ± 4.49 ^a	49.45 ± 4.04 ^{ab}
	UT-10 min + MAP	57.99 ± 1.97 ^a	53.02 ± 1.72 ^a	53.63 ± 2.61 ^a	54.77 ± 3.33 ^a	58.18 ± 4.05 ^a	52.92 ± 5.01 ^a
	UT-15 min + MAP	57.99 ± 1.97 ^a	52.98 ± 1.89 ^a	52.59 ± 1.86 ^a	54.62 ± 2.01 ^a	57.21 ± 3.13 ^a	47.59 ± 3.97 ^b
a^*	Control	-14.85 ± 1.14 ^a	-18.78 ± 0.91 ^c	-17.92 ± 1.11 ^d	-15.88 ± 2.33 ^a	-16.18 ± 2.73 ^b	-15.05 ± 0.64 ^{ab}
	MAP	-14.85 ± 1.14 ^a	-18.07 ± 0.64 ^{bc}	-17.23 ± 0.96 ^{cd}	-16.44 ± 0.55 ^a	-16.37 ± 2.84 ^b	-14.12 ± 2.76 ^{ab}
	UT-5 min + MAP	-14.85 ± 1.14 ^a	-15.04 ± 0.56 ^a	-15.76 ± 0.68 ^{ab}	-15.46 ± 1.49 ^a	-11.45 ± 4.04 ^a	-13.30 ± 2.31 ^a
	UT-10 min + MAP	-14.85 ± 1.14 ^a	-15.73 ± 1.20 ^a	-14.65 ± 1.70 ^a	-16.46 ± 1.01 ^a	-16.68 ± 3.81 ^b	-17.31 ± 1.16 ^c
	UT-15 min + MAP	-14.85 ± 1.14 ^a	-17.45 ± 0.90 ^b	-16.18 ± 1.16 ^{bc}	-16.32 ± 0.97 ^a	-15.67 ± 2.74 ^b	-15.22 ± 1.16 ^b
b^*	Control	19.21 ± 2.22 ^a	29.38 ± 2.73 ^a	27.35 ± 2.78 ^a	29.26 ± 1.99 ^a	35.03 ± 5.86 ^a	30.24 ± 3.05 ^a
	MAP	19.21 ± 2.22 ^a	25.68 ± 2.18 ^b	24.77 ± 2.93 ^{ab}	26.00 ± 1.99 ^b	33.15 ± 4.04 ^{ab}	29.15 ± 1.85 ^a
	UT-5 min + MAP	19.21 ± 2.22 ^a	20.89 ± 1.17 ^c	21.36 ± 1.81 ^c	26.33 ± 2.94 ^b	30.15 ± 1.39 ^{bc}	29.61 ± 5.33 ^a
	UT-10 min + MAP	19.21 ± 2.22 ^a	21.28 ± 2.67 ^c	20.71 ± 3.17 ^c	23.21 ± 3.74 ^c	28.66 ± 3.49 ^c	17.48 ± 4.29 ^b
	UT-15 min + MAP	19.21 ± 2.22 ^a	25.91 ± 2.05 ^b	24.14 ± 2.95 ^b	26.50 ± 2.83 ^b	30.10 ± 4.76 ^{bc}	27.04 ± 3.64 ^a

Values are means of three replicates and standard deviations. Data points in the same column with different letters are significantly different ($P < 0.05$)

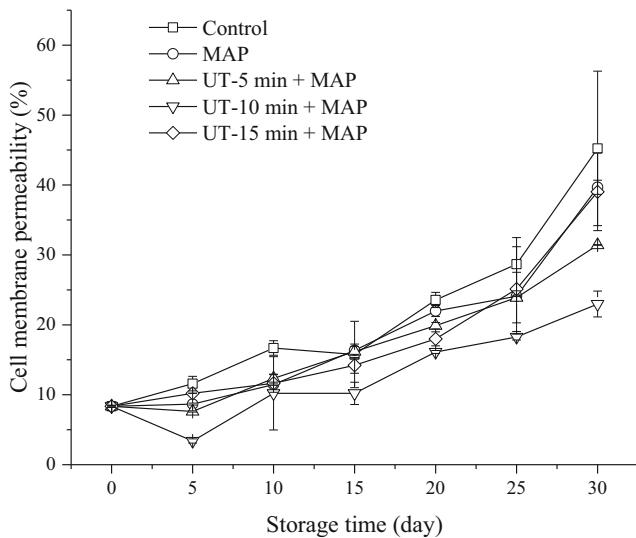


Fig. 3 Changes of the cell membrane permeability of pakchoi during the refrigerated storage. Values are the means of three replicates, and bars stand for standard deviations

and nucleic acids to change the configurations of those macromolecules. MDA can additionally create cross-linking reactions with such molecules and hence the losses of their biological functions. MDA also relaxes the bonds between cellulose molecules and can inhibit the synthesis of proteins (Cárdenas-Coronel et al. 2015). Therefore, accumulation of MDA has a damaging effect on the plasma membrane and organelles of fruits and vegetables.

Figure 4 shows the changes of the MDA content of pakchoi during storage. The MDA content was $9.47 \mu\text{mol/kg} \pm 2.38 \mu\text{mol/kg}$ at the beginning of the storage and increased to $19.45 \mu\text{mol/kg} \pm 0.17 \mu\text{mol/kg}$ in the cases of the control, $18.37 \mu\text{mol/kg} \pm 2.71 \mu\text{mol/kg}$ in the case of MAP, $16.64 \mu\text{mol/kg} \pm 0.75$

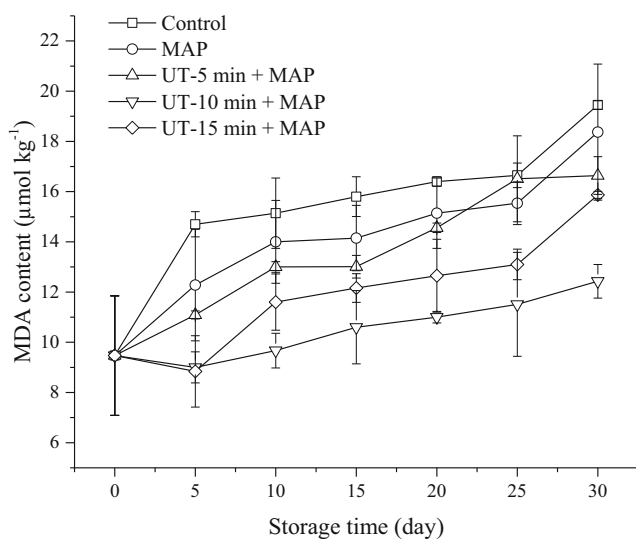


Fig. 4 Changes of the MDA content of pakchoi during the refrigerated storage. Values are the means of three replicates, and bars stand for standard deviations

$\mu\text{mol/kg}$ in the case of UT-5 min + MAP, $12.43 \mu\text{mol/kg} \pm 0.67 \mu\text{mol/kg}$ in the case of UT-10 min + MAP, and $15.87 \mu\text{mol/kg} \pm 0.14 \mu\text{mol/kg}$ in the case of UT-15 min + MAP at the end of the storage. The MDA contents of the samples subjected to UT-5 min + MAP, UT-10 min + MAP, and UT-15 min + MAP were significantly ($P < 0.05$) lower than those of the control and the sample subjected only to MAP at the end of the storage. This demonstrates that ultrasonic treatment in combination with MAP could delay the accumulation of MDA in pakchoi during storage. This may be attributed to the fact that ultrasonication could help decrease the degree of lipid peroxidation and hence reduce the content of MDA (Santos et al. 2015). Compared to values at the beginning of the storage, the MDA content of the sample subjected to UT-10 min + MAP increased by 31.26%, which was about twofold lower than the increase in the content of the control. Li et al. (2017) also reported that ultrasonic treatment for 10 min at 95% relative humidity could decrease the accumulation of MDA in mushrooms.

POD and PPO Activities in Pakchoi During Storage

Enzymatic browning is one of the most significant problems during preservation of fruits and vegetables (O'Donnell et al. 2010). POD is oxidoreductase and a physiological index of maturation and senescence of fruits and vegetables. It can catalyze the oxidation of phenolic compounds, glutathione, and ascorbic acid, creating discoloration (Santos et al. 2015). Changes of the POD activity of pakchoi throughout the storage period are shown in Fig. 5a. The activity of POD showed a tendency to decrease after a short period of increase. The POD activity of UT-10 min + MAP was noted to be significantly ($P < 0.05$) lower than that of control. Ultrasonic treatment for 10 min in combination with MAP could effectively reduce the POD activity of pakchoi.

PPO can catalyze the oxidation of simple phenolic compounds to form terpenoids, which can be further polymerized to form brown- or black-colored polymeric pigments (Fang et al. 2007). In the process of ripening, aging, and storage of postharvest fruits and vegetables, tissue browning is closely related to the PPO activity. Changes of the PPO activity of pakchoi throughout the storage period are shown in Fig. 5b. Ultrasonic treatment in combination with MAP could help inactivate PPO activity, and the sample subjected to UT-10 min + MAP exhibited the lowest PPO activity after storage for 15 days.

The aforementioned inactivation was mainly attributed to the effect of ultrasonic cavitation bubble collapse, which resulted in an extreme increase in localized pressure and temperature, helping to inactivate the enzymes. Acoustic shock waves resulted from such a collapse may also cause strong shear and cause protein denaturation, leading to the breakdown of hydrogen bonding and van der Waals interactions in the polypeptide chains and thus the inactivation of POD and PPO (Başlar and Ertugay 2013; Mawson et al. 2011).

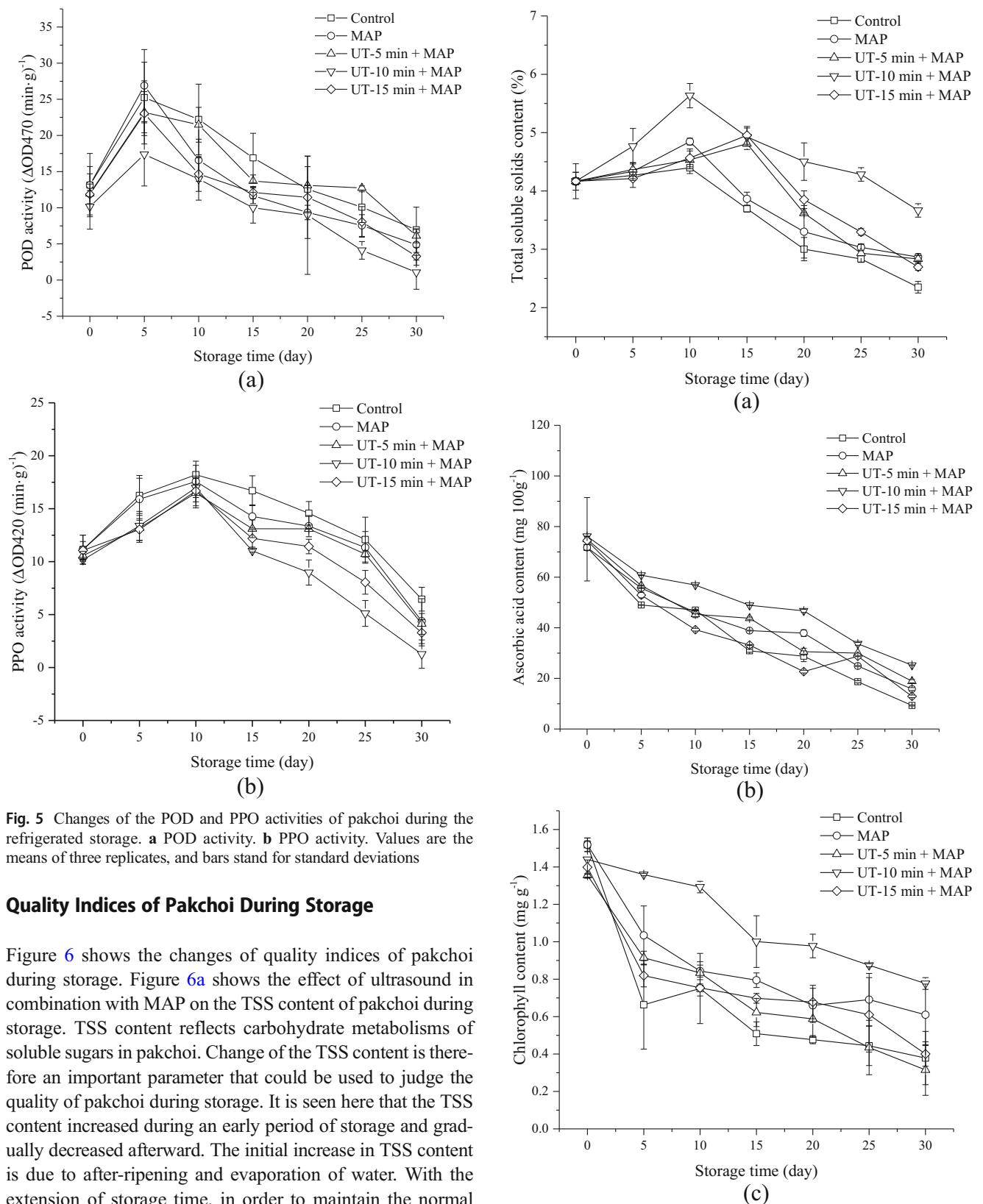


Fig. 5 Changes of the POD and PPO activities of pakchoi during the refrigerated storage. **a** POD activity. **b** PPO activity. Values are the means of three replicates, and bars stand for standard deviations

Quality Indices of Pakchoi During Storage

Figure 6 shows the changes of quality indices of pakchoi during storage. Figure 6a shows the effect of ultrasound in combination with MAP on the TSS content of pakchoi during storage. TSS content reflects carbohydrate metabolisms of soluble sugars in pakchoi. Change of the TSS content is therefore an important parameter that could be used to judge the quality of pakchoi during storage. It is seen here that the TSS content increased during an early period of storage and gradually decreased afterward. The initial increase in TSS content is due to after-ripening and evaporation of water. With the extension of storage time, in order to maintain the normal physiological and metabolic activities of pakchoi, respiration occurred and nutrients were consumed, resulting in the decrease in TSS content, which indeed reflects the senescence of the vegetables (Brummell and Harpster 2001).

Fig. 6 Changes of the quality indices of pakchoi during the refrigerated storage. **a** Changes of total soluble solids. **b** Changes of ascorbic acid content. **c** Changes of chlorophyll content. Values are the means of three replicates, and bars stand for standard deviations

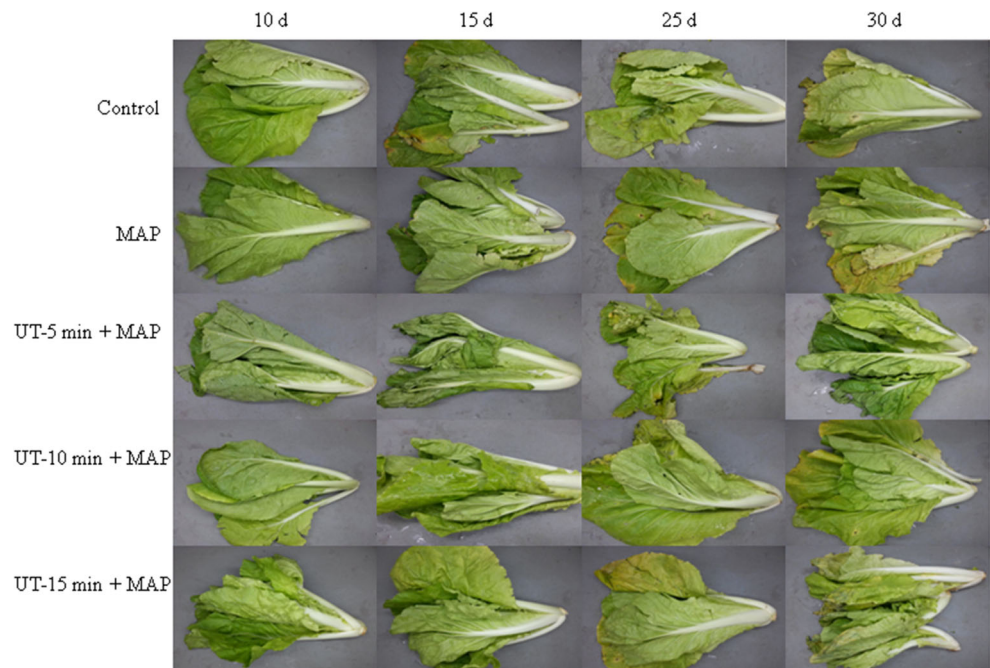
TSS contents of pakchoi which undergone different ultrasonic treatments were higher than those of the control. The TSS content of pakchoi subjected to UT-10 min + MAP was significantly ($P < 0.05$) higher than that of the other samples. This shows that appropriate application of ultrasound could help delay the degradation of soluble solids in pakchoi. Such an observation may be due to the cavitation and mechanical effect of ultrasound, which helped limit enzymatic hydrolysis of carbohydrates (Islam et al. 2014). Application of ultrasound creates free radicals such as H^+ and OH^- as a result of decomposition of water within vegetable cell cavities. This, in turn, results in an increase in the acidity, which helped limit the hydrolysis of sugars (Cruz Cansino et al. 2013; Pandey 2011). The lowest TSS content of the control might be due to spoilage and fermentation of the vegetables as a result of the conversion of sugars to acids, carbon dioxide, or alcohol (Costa et al. 2013). Fan et al. (2019) indeed reported similar results that ultrasonic treatment for 10 min could effectively maintain the TSS content of fresh-cut cucumber during storage.

Ascorbic acid is of importance to demonstrate the nutritional quality of fruits and vegetables (Lu et al. 2010; Miller and Rice-Evans 1997). Changes of ascorbic acid content of pakchoi treated with ultrasound in combination with MAP during storage are shown in Fig. 6b. At prolonged storage period, ascorbic acid content showed a decreasing tendency. Compared with that of the control, the ascorbic acid content of pakchoi subjected to UT-10 min + MAP was noted to be higher. It could be inferred then that ultrasonic treatment could help reduce the loss of ascorbic acid and preserve the nutritional value of pakchoi. After storage for 30 days, the ascorbic acid contents of the control as well as samples subjected to MAP, UT-5 min + MAP, UT-10 min +

MAP, and UT-15 min + MAP were 9.29 mg/g, 15.78 mg/g, 18.88 mg/g, 25.21 mg/g, and 12.89 mg/g, respectively; these represent the decrease of 87.05% for the control, 78.00% for the MAP sample, 74.82% for the UT-5 min + MAP sample, 66.89% for the UT-10 min + MAP sample, and 82.67% for the UT-15 min + MAP sample when compared with the value at the beginning of the storage. The sample subjected to UT-10 min + MAP exhibited the highest retention rate of ascorbic acid compared to the other samples. This may be due to the fact that ultrasound created cavitation effect, thus lowering the content of dissolved oxygen, which is an important factor in ascorbic acid degradation (Bhat et al. 2011). Cao et al. (2010) revealed the similar results that ultrasonic treatments at 40 kHz and 59 kHz could help preserve ascorbic acid in strawberries.

Figure 6c shows the changes of the chlorophyll content of pakchoi during storage. Chlorophyll content can reflect the color change of leafy vegetables during storage and preservation and hence is a good index to judge the yellowing rate of pakchoi. Chlorophyll content exhibited a decreasing trend in all cases. Nevertheless, the chlorophyll content of the sample subjected to UT-10 min + MAP was significantly ($P < 0.05$) higher than that of the control. After storage for 30 days, the chlorophyll content of UT-10 min + MAP was $0.78 \text{ mg/g} \pm 0.03 \text{ mg/g}$, while those of the control and the sample subjected to MAP were $0.38 \text{ mg/g} \pm 0.14 \text{ mg/g}$ and $0.61 \text{ mg/g} \pm 0.16 \text{ mg/g}$, respectively. The chlorophyll contents of the samples subjected to UT-5 min + MAP and UT-15 min + MAP were $0.31 \text{ mg/g} \pm 0.14 \text{ mg/g}$ and $0.40 \text{ mg/g} \pm 0.07 \text{ mg/g}$, respectively. The results here illustrate that ultrasound could help retain chlorophylls, and UT-10 min + MAP is the optimal treatment condition. Cao et al. (2010) indeed mentioned the maintenance of ascorbic acid and

Fig. 7 Photographs of pakchoi during the refrigerated storage



chlorophylls as a result of ultrasonic treatment, which helped inhibit respiration of a vegetable during its storage.

Photographs of pakchoi during the storage are shown in Fig. 7. Pakchoi treated with UT + MAP appeared better than the control and that undergone MAP alone. After the 30-day storage, the sample subjected to UT-10 min + MAP exhibited the best leaf quality, with a minimum leaf-yellowing area, the lowest degree of wilting, and the brightest green color.

Conclusions

Based on the results on the total number of colonies, mass loss, color change, cell membrane permeability, MDA content, and POD and PPO activities as well as selected quality indices, viz. TSS as well as ascorbic acid and chlorophyll contents, it is shown here that pakchoi which undergone ultrasonic treatment in combination with MAP maintained its quality better than the control and that undergone only MAP during 30 days of refrigerated storage. Among the ultrasonically treated samples, the application of ultrasound for 10 min significantly ($P < 0.05$) delayed the increase in mass loss, color indices, and accumulation of MDA and reduced the increasing rate of cell membrane permeability, as well as POD and PPO activities. The TSS content as well as ascorbic acid and chlorophyll contents of pakchoi also suffered less changes in the case of a sample subjected to UT-10 min + MAP. We will certainly continue our study on the effect of initial gas composition on the physiology and quality of packaged pakchoi to arrive at a more appropriate gas composition for this produce.

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