



Enhancement for Microbial Safety of Peeled Shallot (*Allium ascalonicum* L.) by the Application of Hot Water and Acidified Sodium Chlorite

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Abstract

Shallot (*Allium ascalonicum* L.) is one of the most used ingredients that is commonly found in Asian cuisine preparation. However, it poses a safety risk due to the microbial contamination. The ideal conditions for peeled shallot processing were pre-treated with hot water (HW) followed by 100 ppm acidified sodium chlorite (ASC) solution and packed under vacuum packaging (VP), then stored at $5\pm 2^\circ\text{C}$. This condition reduced the loads of aerobic bacteria, yeasts and molds during storage by 0.68-0.80 and 0.46-0.95 log CFU/g FW, respectively, which was better than the control samples. There was a slight increase in weight loss and total phenolic content during cold storage. The combination treatments of HW and ASC packed under VP had no effect on weight loss and antioxidant capacity as compared to control sample throughout the storage period.

Introduction

Shallot (*Allium ascalonicum* L.) is an edible plant that belongs to the family Alliaceae, which is similar to the onion (*Allium cepa* L.). The common name of shallots are bulbous and herbaceous plants. Shallot is a popular food ingredient due to it has a medicinal effects, distinct aroma and flavor (Dron et al., 1997). Moreover, it has a good source of antioxidants and antimicrobial properties (Leelarungrayub et al., 2006; Raeisi et al., 2016). The

“minimally processed”, “slightly processed”, “ready to eat”, and “fresh-cut” produces has increased in popularity in consumer demand (Cantwell & Suslow, 2002). However, processing techniques of fresh-cut fruits and vegetables such as washing, selecting, decorating, peeling, cutting and chopping does not impact the “fresh-like” quality of the fresh-cut produce. The shelf-life of fresh-cut produce generally declines due to the undesirable effects of physiological and biochemical changes as well as an increase of the microbial

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population. The peeling and cutting surfaces of the fruits and vegetables induce quality loss and microbial contamination rendering the products unmarketable due to their undesirable appearance. Several technologies are used to extend the shelf life of fresh-cut fruits and vegetables. For example, chlorinated water has been used extensively to eliminate foodborne pathogens and spoilage microorganisms in fresh-cut products (Guo et al., 2017; Huang & Chen, 2018). However, the use of chlorinated water raises concerns regarding the environmental and health implications of halogenated by-products such as trihalomethanes and chloramines (Ölmeza & Kretzschmar, 2009). Therefore, the alternative method has also been used to monitor undesirable physiological changes and prolong the shelf life of fresh-cut products. Especially, thermal or heat treatments have been used to control the microbial populations and extend the shelf life of the product.

Hot water (HW) treatment is a chemical-free method for reducing foodborne pathogens, delaying senescence (Dea et al., 2010; Siddiq et al., 2013; Kabelitz & Hassenberg, 2018) and inhibition of browning reaction in fresh-cut produce (Wang et al., 2014). Acidified sodium chlorite (ASC) is an effective and efficient oxidizing agent that is an alternative disinfectant to chlorine (Cruz et al., 2006). According to the Food and Drug Administration (FDA), the application of ASC is able to generate chlorine dioxide gas, which can be used as an antimicrobial agent for disinfecting water and washing fruits, vegetables and poultry (FDA, 2010). ASC is produced by decreasing the pH (2.5–3.2) of sodium chlorite (NaClO_2) solution with any acid that has a Generally Recognized as Safe (GRAS) status such as citric acid (FDA, 2000). The FDA has approved the use of 500–1200 ppm ASC for spraying and dipping fresh and fresh-cut produces. ASC is an antibiotic agent that prevents browning reaction in various minimally processed fruits and vegetables such as cilantro (Allende et al., 2009), pears (Xiao et al., 2011), broccoli (Renumarn et al., 2015) and rose apple (Mola et al., 2016). Furthermore, ASC does not produce carcinogenic compounds when compared with chlorine (Cruz et al., 2006). However, the effective approaches for preventing microbial contamination and maintaining the quality of peeled shallot have rarely been investigated.

Therefore, the purpose of this study was to investigate the effect of HW pre-treatment with/without ASC solution on peeled shallot processing methods to minimize the microbial loadings. Passive packaging (PP)

and vacuum packaging (VP) were used to maintain the quality and antioxidant capacity of peeled shallot throughout the refrigeration storage.

Materials and methods

1. Plant material and treatment

Shallots (*Allium ascalonicum* L.) were purchased from the local marketplace in Prachinburi province, Thailand. They were selected by considering the absence of infected, damaged, and the uniformity of color, shape, and size. They were stored at room temperature ($25 \pm 2^\circ\text{C}$) until the time of use in the experiments. Shallots were blanched in boiled water ($95 \pm 2^\circ\text{C}$) for 30 seconds, and then immediately cooled down to $20 \pm 2^\circ\text{C}$ with tap water. After that, they were immersed in 100 ppm acidified sodium chlorite solution (pH 4, citric acid) for 10 min, followed by rinsing with drinkable water. The excess water of peeled shallot was removed with sterile tissue paper. Approximately 150 ± 5 g of minimally processed shallots were packed in a passive packaging (PP), or under vacuum packaging (VP) of polyethylene (PE) bags (150×200 mm, $30 \mu\text{m}$ thickness). The packed samples were stored at $5 \pm 2^\circ\text{C}$ for 9 days. Quality attributes (weight loss), total phenolic content, antioxidant properties, and microbial populations were evaluated every three days of storage. Each treatment had three replicates (bags).

2. Microbiological analysis

Total aerobic bacteria and fungi (yeasts and molds) counts were analyzed using a spread plate method according to Renumarn & Choosuk (2020) with minor modifications. Briefly, 25 g peeled shallot sample was transferred into a stomacher bag, which contained 225 mL of 0.1% sterile peptone solution (Peptone, HiMedia, India), and homogenized for 1 min with a stomacher (Stomacher® 400 Circulator, UK). The various serial dilutions of each homogenized samples were dispersed on an agar plate. Total aerobic bacteria were enumerated on Plate Count Agar (PCA, HiMedia, India), which was incubated at 35°C for 48 h. Yeasts and molds were enumerated on Potato Dextrose Agar (PDA, HiMedia, India), which were incubated at 28°C for 7 days. Microbial populations were then enumerated by counting on agar plates that contained 25–250 colonies and microbial population were expressed as log colony forming units (CFU) /g fresh weight (FW) of shallot sample.

3. Determination of weight loss

Each shallot bags were assessed for the percentage of weight loss, by comparing the weight of samples at each sampling date and their initial weight (150 ± 5 g).

4. Sample extraction

Approximately 10 g of peeled shallots were homogenized with 100 mL of 90% ethanol before being centrifuged at 9,000 g at 4°C for 20 min. The supernatants were collected for analysis of total phenolic content and antioxidant activity.

5. Determination of total phenolic content

Phenolic content was determined using the Folin-Ciocalteu method (Roy et al., 2009) with slight modifications. Briefly, 0.4 mL of sample extract was mixed with 2 mL of 10% Folin-Ciocalteu's phenol reagent, vortexed, and placed at $25 \pm 5^\circ\text{C}$ for 4 min. The mixture was mixed with 1.6 mL of 5% (w/v) sodium carbonate solution and incubated at $25 \pm 5^\circ\text{C}$ in the dark for 60 min. The absorbance was measured at 765 nm using a spectrophotometer. A calibration curve of gallic acid standards (0, 20, 40, 60, 80 and 100 mg/L) was used to determine the phenolic content of the samples, which were expressed as mg of gallic acid equivalents (GAE)/g fresh weight (FW).

6. Determination of antioxidant activity

The antioxidant activity was assayed by measuring the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) radical as described by Brand-Williams et al. (1995) and Thaipong et al. (2006), with some modifications. Briefly, 0.2 mL of sample extract was mixed with 0.6 mL of 0.075 mM DPPH[•] and 5.2 mL of 95% ethanol mixed and incubated for 30 min in the dark. The absorbance was measured at 517 nm using a spectrophotometer. The percentage of DPPH[•] radical scavenging activity was estimated following the equation below:

$$\text{DPPH}^{\bullet} \text{ radical scavenging (\%)} = (\text{Abs (control)} - \text{Abs (sample)}) / (\text{Abs (control)}) \times 100$$

7. Statistical analysis

The results of all experiments were expressed in the term of mean \pm standard deviation (SD). The significant difference between the mean values was estimated by analysis of variance (ANOVA), followed by the Duncan's multiple range test. Statistically significant differences were informed as $p \leq 0.05$. SPSS software was used for all statistical analyses (SPSS version 21.0, SPSS Inc., Chicago, IL., USA).

Results and discussion

1. Effects of pre-treatment on microbial populations

The most important step in the minimally process of fresh-cut production during refrigeration storage are the quality of microbial loads, and sensory quality of fresh-cut produce (Artés & Allende, 2005). Microbial quality assessments also suggested that the produce was acceptable at a safe level. In this research, the results showed that pre-treatment with HW followed by ASC solution for 10 min, and packed under VP could reduce aerobic bacteria, yeast and molds counts during storage at $5 \pm 2^\circ\text{C}$ (Fig. 1A). At the initial day, HW pre-treatment with ASC solution had significantly ($p \leq 0.05$) reduced aerobic bacteria counts of peeled shallot, by 0.71 log CFU/g FW as compared to HW treatment alone. Hence, the treatment of HW pre-treatment with ASC solution, and packed under VP showed the greatest significant reduction in aerobic bacteria counts (0.68-0.80 log CFU/g FW) of peeled shallots during storage as compared to the other treatments (Fig. 1A). However, the counts of aerobic bacteria slightly increased in all samples until the end of storage. At the 9th day of storage, the lowest count of aerobic bacteria was found in the peeled shallot sample treated with HW, followed by ASC, and packed under VP (4.57 log CFU/g FW), whereas it was 5.25 log CFU/g FW in the peeled shallot sample treated with HW, and packed in PP. Similarly, many previous research have shown that the application of ASC could reduce the microbial growth in various fresh-cut produce. For example, Martínez-Sánchez et al. (2006) found that ASC at the concentration of 250 ppm could reduce pathogenic bacterial growth in rocket leaves. Moreover, Allende et al. (2009) suggested that the use of 250 or 500 ppm of ASC could reduce microbial populations in fresh-cut cilantro.

The peeled shallots treated with HW pre-treatment had an initial yeast and molds count of 1.62 log CFU/g FW, whereas it was 1.16 log CFU/g FW in the samples treated with HW pre-treatment, followed by ASC solution, and packed in both PP and VP (Fig 1B). At the 9th day of storage, the lowest count of yeasts and molds was found in the sample treated with HW, followed by ASC, and packed under VP (3.35 log CFU/g FW), whereas it was 3.84 log CFU/g FW in the sample treated with HW, followed by ASC, and packed in PP, which were similar to aerobic bacteria counts (Fig. 1A). These results indicated that the pre-treatment with HW, followed by ASC solution, and packed under VP could enhance

the control of both aerobic bacteria, yeasts and molds in peeled shallot as compared to the other treatment packed in PP. Commonly, aerobic bacteria, yeasts and molds require O₂ for their growth. Therefore, the products stored under the atmosphere less than 1% O₂ (vacuum packaging) could retard the growth of aerobic microorganisms as compared to air atmosphere packaging (Masniyom, 2011).

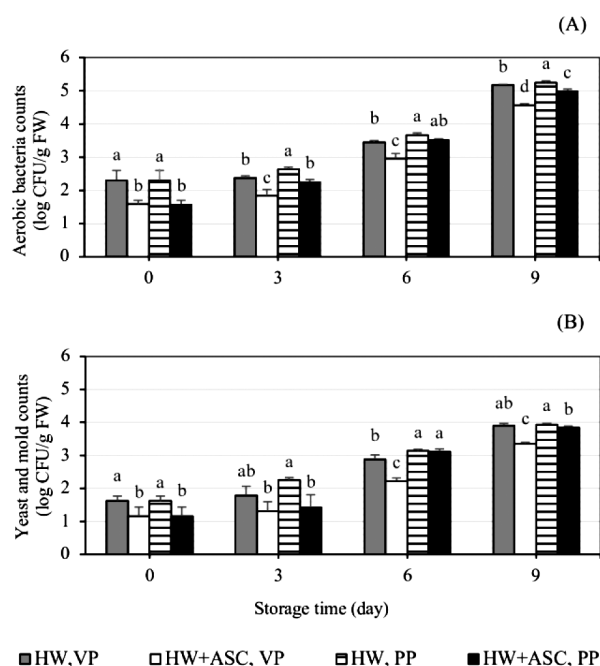


Fig. 1 Effects of pre-treatments on aerobic bacteria count (A) and yeast and mold count (B) of peeled shallots during storage at 5±2°C (HW, hot water; ASC, acidified sodium chlorite; VP, vacuum packaging; PP, passive packaging). The data represent mean values ± SD (n = 3). The various small letters at the top of the bars in each day indicate significant differences ($p \leq 0.05$)

2. Change of weight loss (%)

Fresh-cut fruits and vegetables are highly perishable products that are associated with respiration, transpiration, surface dehydration, as well as the release of heat and loss of water, due to the processing of fresh-cut produces, e.g. trimming, peeling, grading, cutting and shredding, etc. (Zhang et al., 2019). In this research, weight loss of all peeled shallot samples storage at 5±2°C slightly increased over the time of storage, with ranges of 0.070–0.743% (Table 1). At the 3rd day of storage, peeled shallot sample treated with HW, and packed under VP had the highest percentage of weight loss (0.743%) with significantly difference ($p \leq 0.05$) was found when compared to the other treatments. However, no significant

difference in weight loss were found in all samples ($p > 0.05$) at the 6th and the 9th day of storage. After storage for 9 days, peeled shallots treated with HW, with/without ASC, and packed under VP showed the minimum weight losses (0.183 and 0.240%, respectively) as compared to those samples packed in PP (0.390 and 0.627%, respectively). The weight loss of the vacuum-packed shallot samples during cold storage was in the highest range of 0.280–0.743%, during the first period of storage. After 6 days of storage, the weight loss was reduced to 0.183–0.240%, resulting greater in samples packed under vacuum condition. The type of packaging and packing method also affected the moisture inside the package and percentage of weight loss (Chang & Kim, 2015; Reche et al., 2019). Similarly, previous studies have found that the fruits packed in vacuum packaging increased the moisture inside the package and reduced weight loss during storage (Chang & Kim, 2015; Moradinezhad & Dorostkar, 2021). Weight loss is one of the important quality attribute that could be used to measure the shelf life of fresh-cut fruits and vegetables, which can lead to the retail value of the entire producer (Rivera-López et al., 2005; Loi et al., 2019).

Table 1 Effects of pre-treatments on the change of weight loss (%) in peeled shallots during storage at 5±2°C

Treatment	Weight loss (%)		
	Day 3	Day 6 ^{ns}	Day 9 ^{ns}
HW, VP	0.743±0.44 ^a	0.733±0.07	0.240±0.21
HW+ASC, VP	0.280±0.26 ^{ab}	0.363±0.04	0.183±0.16
HW, PP	0.207±0.01 ^b	0.137±0.24	0.627±0.88
HW+ASC, PP	0.070±0.35 ^b	0.173±0.21	0.390±0.34

Remark: The averages followed by the different letter in each column indicates that there are significant differences at $p \leq 0.05$. ns, not significantly differences; HW, hot water; ASC, acidified sodium chlorite; VP, vacuum packaging; PP, passive packaging

3. Effects of pre-treatment on total phenolic content

Total phenolic content of all samples slightly increased with an increase in storage period at 5±2°C (Fig. 2A). On the initial day of storage (day 0), all peeled shallot samples had total phenolic content of 87.94–88.71 mg GAE/g FW. After the 9th day of storage, all peeled shallot sample had a high total phenolic content, which were in the range of 95.80–106.72%, except for the sample treated with HW, and packed under VP showed significantly the lowest ($p \leq 0.05$) total phenolic content (63.85%) that was found between the treatment conditions. This result may be associated with the induction of abiotic stress in plants metabolism when oxygen depleted in the packaging during cold storage.

These results also may indicate that the total phenolic content decreased depending on the O_2 level inside the package. Total phenolic contents of shallot in this experiment were higher than those of shallot (*Allium oschaninii* L.) in different cultivars (17.18 mg GAE/g FW) (Lu et al., 2011). According to Siddiq et al. (2013), plants stress from wounding and cutting can lead to an increase of phenolic compounds synthesis. However, improving the efficiency of postharvest handling, and the selection of suitable packaging could maintain the phenolic content of the fresh and fresh-cut produce.

4. Effects of pre-treatment on antioxidant activity

The antioxidant activity measured by the DPPH radical scavenging. At the initial day of storage, the antioxidant activity of all peeled shallot samples were in the range of 18.58-22.80% (Fig. 2B). During storage at $5\pm 2^\circ\text{C}$, the antioxidant activity of all samples tended to slightly increase as well as total phenolic content (Fig. 2A). The antioxidant activity of peeled shallot samples packed in VP, found 18.58-25.77% (only HW) and 22.80-24.18% (HW+ASC), respectively. Whereas, the pre-treated sample packed in PP, found 18.58-25.55% (only HW) and 22.80-24.37% (HW+ASC), respectively (Fig. 2B). There was no significant ($p>0.05$) difference in the antioxidant activity among treatment conditions. Our results show that the application of peeled shallot samples treated with HW, followed by ASC and packed under VP did not affect the loss of antioxidant activity during storage at $5\pm 2^\circ\text{C}$. Similar results were observed by Zudaira et al. (2020), who suggested that fresh-cut calçots (*Allium cepa* L.) stored under vacuum packaging preserved the physicochemical quality better than passive modified atmosphere after 15 days. Nevertheless, previous research has demonstrated that fresh and fresh-cut produce could be packed in the high-oxygen atmosphere ($O_2\geq 60\%$) to enhance the antioxidant capacity as reported in blueberry fruit (Zheng et al., 2003). Furthermore, Selcuk & Erkan (2015) reported that the decrease of antioxidant activity was found in sour-sweet pomegranates cv. Hicaznar when stored under low or absent O_2 conditions in modified atmospheres packaging (MAPs). Pinela et al. (2016) also reported that the best treatment for maintaining the DPPH scavenging activity of fresh-cut watercress samples was air packaging.

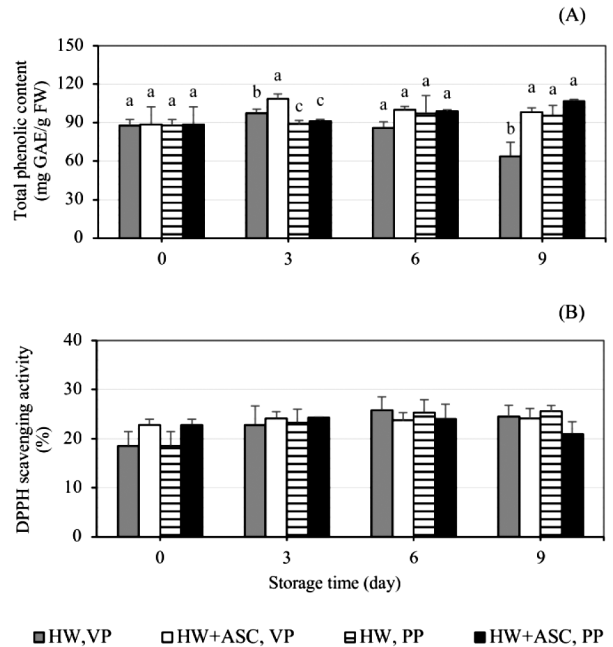


Fig. 2 Effects of pre-treatments on total phenolic content (A) and DPPH scavenging activity (B) of peeled shallots during storage at $5\pm 2^\circ\text{C}$ (HW, hot water; ASC, acidified sodium chlorite; VP, vacuum packaging; PP, passive packaging). The data represent mean values \pm SD ($n=3$). The various small letters at the top of the bars in each day indicate significant differences ($p\leq 0.05$)

Conclusion

The combination pre-treatment with HW, followed by 100 ppm ASC solution, and packed under VP had effectively inhibit the microbial growth, maintaining the quality and antioxidant capacity of peeled shallot during cold storage. This condition could be used to prolong the shelf life of peeled shallot. Therefore, these results could be a promising procedure to extend its application for maintaining the qualities of the plant in the family Alliaceae such as onion, garlic, leek, scallion, chive and garlic chives, etc.

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