Plasma-Activated Water Enhances the Efficacy of Chlorite Treatments Against Browning and Fungal Growth in Aromatic Coconut Mesocarp

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Abstract

Aromatic coconuts are commonly trimmed to enhance their appearance and market value. However, the trimming process often induces enzymatic browning and fungal contamination of the mesocarp surface. This study investigated the effect of sequential plasma-activated water (PAW) and chlorite-based treatments on browning and fungal growth in aromatic coconut mesocarp. Coconut mesocarps (4.0 × 4.5 cm) were dipped for 5 min in PAW, followed by sodium chlorite (SC) or acidified sodium chlorite (ASC) for 5 min. Filtered water (FW) and sodium metabisulfite (SMS) served as controls. All samples were stored at 4℃ for 9 days. Results showed that PAW followed by SC and PAW followed by ASC effectively inhibited browning. Notably, PAW followed by ASC significantly reduced fungal growth, whereas PAW followed by SC did not. These findings suggest that sequential PAW-ASC treatment has potential for controlling browning and fungal contamination in coconut mesocarp. However, further studies are needed to validate and optimize the treatment conditions in commercial applications.

Keywords: Plasma technology, Sodium chlorite, Acidified-sodium chlorite, Fungal growth, Enzymatic browning

Introduction

Aromatic coconut (*Cocos nucifera* L.) belongs to the family Arecaceae and the subfamily Cocoideae (Lopez, 2023). It is widely consumed as a fresh beverage, and Thailand is one of the main exporters of aromatic coconuts to international markets. To reduce weight during transportation and enhance the visual appearance of aromatic coconut, the mesocarp is typically trimmed to form a conical top, a flat base, and a cylindrical body. However, trimming causes tissue injury, leading to the release of phenolic compounds that, in the presence of oxygen, are oxidized by polyphenol oxidase (PPO), resulting in melanin formation and surface browning (Walker and Wilson, 1975; Friedman, 1996). The exposed nutrient-rich surface also promotes microbial growth. Both problems represent the main physiological disorders reducing the sensory quality and shelf life of trimmed aromatic coconut.

Traditionally, sodium metabisulfite (SMS) has been used to control browning and microbial contamination. However, SMS has been banned in some countries due to health risks, including asthma and skin irritation in sulfite-sensitive consumers (Lien et al., 2016). With increasing concerns about food safety, alternative additives and non-thermal treatments have gained considerable attention. Compounds such as sodium chlorite (SC) and acidified sodium chlorite (ASC) have demonstrated potential to inhibit enzymatic browning and microbial growth in various fresh and fresh-cut products. Both chemicals have been approved by the U.S. Food and Drug Administration for dip or spray application on food items, including fresh and fresh-cut fruits and vegetables (Anonymous, 2000), with solutions used for sanitizing shall not exceed 200 ppm of available chlorine (U.S. Food and Drug Administration, 1998). Previous studies have shown that SC significantly inhibited browning in fresh-cut Red Delicious apples (Luo et al., 2011), and ASC effectively reduced browning on apple slices (Lu et al., 2006).

Plasma-activated water (PAW) is produced by treating water with a stream of ionized gas (plasma) (Wong et al., 2023). This process increases the oxidation-reduction potential (ORP), lowers pH, and enhances conductivity due to the transfer of reactive

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species from plasma to water. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated in PAW can disrupt the intramolecular bonds of peptidoglycan by removing a hydrogen atom from the peptide bond in the cell walls, leading to wall degradation, metabolic arrest, cell damage, and ultimately death (Wong et al., 2023). In recent years, some studies have reported the effectiveness of PAW in inactivating pathogenic microbes and maintaining the quality of produce. Chen et al. (2019) reported that PAW significantly inhibited the growth of aerobic bacteria, yeast, and mold, and helped maintain the quality of fresh-cut pears during storage. Similar results have been reported that PAW exhibits strong antimicrobial activity on fresh produce, such as grapes, goji berries, and shiitake mushrooms, without causing significant adverse effects (Cong et al., 2022; Gavahian et al., 2020; Guo et al., 2017). The use of PAW has thus emerged as a promising alternative to conventional sanitizing methods in food systems. However, no reports have examined the combined application of food additives such as sodium chlorite (SC) or acidified sodium chlorite (ASC) for inhibiting browning and fungal growth in trimmed aromatic coconuts. Therefore, this study aimed to explore the potential of PAW to enhance the efficacy of SC or ASC against browning and fungal growth in the mesocarp tissue, with the ultimate goal of maintaining the quality of trimmed aromatic coconuts.

Material and methods

PAW preparation

PAW was generated in-bound for 20 minutes using a Si-plasma generator (model: APPS52C, Creating Nanotechnology Co. Ltd., Taiwan), operated at 800 W with a clean dry air flow rate of 40 L/min.

Chemical solution preparation

A 3% sodium metabisulfite (SMS) solution was prepared by dissolving 90 g of SMS in 3 liters of distilled water (DW). Sodium chlorite (SC) solution at 200 ppm was prepared by dissolving 600 mg of SC in 3 liters of DW. Acidified sodium chlorite (ASC) solution at 200 ppm was prepared by dissolving 60 mL of 1% sodium chlorite solution in 3 liters of DW, then adjusting the pH to 2.3 by adding 1% citric acid solution.

Determination of physicochemical properties of PAW

The physicochemical properties of PAW were measured using standard analytical instruments. The pH was measured with a pH meter (FiveEasy F20, Mettler Toledo, Switzerland), while electrical conductivity and oxidation-reduction potential (ORP) were measured using an EC meter and ORP meter, respectively. For the determination of hydrogen peroxide (H_2O_2) concentration, 100 µL of PAW was mixed with 1 mL of 50% trichloroacetic acid (TCA), 1 mL of 10 mM ammonium iron (II) sulfate hexahydrate, and 0.5 mL of 2.5 M potassium thiocyanate. The resulting solution was analyzed using a spectrophotometer at 480 nm, and H_2O_2 concentration was expressed in µg/L (Deadman et al., 2017).

Coconut mesocarp preparation

Aromatic coconuts at the stage 1.5 layers of meat (7-8 months after full bloom) were sourced from a farm in Ratchaburi province and transported to the Postharvest Technology Division Laboratory at King Mongkut's University of Technology Thonburi (KMUTT), Bangkok, Thailand. The fruits were selected based on uniformity in size, shape, color, and disease-free. The fruit was washed with tap water and air-dried at ambient temperature. Mesocarp was cut into small rectangular pieces (4.0 × 4.5 cm, 0.5 cm thickness). Coconut mesocarp pieces were dipped in 3 liters of PAW for 5 minutes, followed by SC or ASC for 5 minutes. The mesocarp pieces were dipped for 5 minutes in filtered water, and SMS served as controls. All samples were then placed in a LDPE zipper plastic bag (15 x 23 cm, 0.05 mm thick, 0.5 kg pack, ARO brand, Thailand) and stored at 4°C for 9 days.

Total color difference determination

Color values (L*, a*, and b*) were measured using a colorimeter CR 300 (Minolta, Japan). Colors were taken on days 0, 1, 3, 5, 7, and 9. Samples were measured on the upper and bottom sides. Total color differences (ΔE) were calculated using Equation 1:

$$\Delta E=\sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2} \quad (1)$$

Browning index determination

Browning index (BI) was calculated from L*, a*, b* values using Equations 2 and 3 (Treesuwan et al., 2022):

$$x = \frac{a^*+1.75L^*}{5.645L^*+a^*-3.012b^*} \quad (2)$$

$$BI = \frac{100(x-0.31)}{0.172} \quad (3)$$

Fungal growth analysis

The mesocarp pieces (1.0 × 1.0 cm) were placed onto potato dextrose agar (PDA) containing streptomycin. PDA plates were kept for 7 days at room temperature (27 ± 2°C) (Payuhamaytakul et al., 2018). The percentage of fungal contamination was calculated using Equation 4:

$$\text{Fungal contamination (\%)} = \frac{\text{num. of aromatic coconut pieces shown fungi}}{\text{total of coconut pieces}} \times 100 \quad (4)$$

Experimental design and data analysis

All experiments were performed using a completely randomized design (CRD) with three replications, where each replication consisted of four mesocarp pieces. Data were analyzed via analysis of variance (ANOVA) at a 5% significance level ($p<0.05$) using SAS statistical software. Mean comparisons were conducted using Duncan's multiple range test (DMRT) to identify significant differences between treatments.

Results

Effect of PAW followed by SC or ASC on mesocarp color

As shown in Figure 1A, the L* value of the coconut mesocarp treated with PAW followed by SC showed a slight decrease during storage, similar to the result of PAW followed by ASC. Both treatments are significantly different from the control, which experienced a sharp decrease on day 1. Meanwhile, SMS was the most effective treatment, maintaining the L* value throughout the storage period. These findings suggest that PAW followed by SC or ASC can mitigate surface browning compared with the control, but are still less effective than SMS.

For a* and b* values, the control exhibited sharp increases on day 1, likely due to rapid browning after trimming. In contrast, PAW followed by SC and PAW followed by ASC showed minimal increases and remained relatively stable toward the end of storage. These treatments performed better than the control, with significant differences observed between day 1 to day 7, though they were still less effective than SMS (Figure 1B and 1C). Similarly, the ΔE value of PAW followed by SC and PAW followed by ASC remained relatively stable and showed a slight but significant increase at the end of storage 7 (Figure 1D). The result suggested that PAW followed by SC or ASC can suppress overall color change (ΔE) of coconut mesocarp during storage.

Effect of PAW followed by SC or ASC on browning index

The browning index (BI) shown in Figure 2 indicates that samples treated with PAW followed by SC and PAW followed by ASC exhibited a slight increase in BI during storage, reaching maximum values of 76.88 and 73.86, respectively. Both treatments were significantly different from the control from day 1 to day 7, with the control reaching its highest BI around 117.05 on day 1. In contrast, SMS maintained the lowest BI throughout storage. These results suggest that PAW followed by SC and PAW followed by ASC effectively prevent browning from day 1 to day 7, although they are still less effective than SMS.

Effect of PAW followed by SC or ASC on fungal contamination

Based on Figure 3, PAW followed by ASC reduced fungal contamination to 58%, whereas PAW followed by SC showed no inhibitory effect. However, PAW followed by ASC remained less effective than SMS. The significant reduction in fungal contamination by PAW followed by ASC is likely due to their synergistic effect of PAW and ASC. PAW provides reactive species with antifungal properties (Du et al., 2023; Fan and Wang, 2020), while ASC, characterized by low pH, high electric conductivity (EC), and high oxidation-reduction potential (ORP), further enhances microbial inactivation.

Physicochemical properties of PAW

The physicochemical properties of PAW were significantly different from the filtered water and SMS solution (Table 1). PAW exhibited the lowest pH value (2.79), indicating strong acidity, whereas SMS and filtered water showed higher pH values of 3.86 and 5.76, respectively. The acidification seen in PAW is likely due to the formation of reactive nitrogen species (RNS) and reactive oxygen species (ROS), during plasma activation. Electrical conductivity (EC) of PAW (498.33 mS/cm) was substantially lower than that of SMS (25,500 mS/cm) but higher than filtered water (22.67 mS/cm). In contrast, oxidation-reduction potential (ORP) was markedly elevated in PAW (544.33 mV), suggesting strong oxidizing capacity, while filtered water and SMS recorded much lower values of 219.67 mV and 105.00 mV, respectively. Similarly, PAW also contained the highest hydrogen peroxide (H_2O_2) concentration (5.56 µg/L), more than twice that of the other treatments (filtered water at 2.11 µg/L and SMS at 1.84 µg/L). These results confirm that the increased production of H_2O_2 in PAW aligns with the elevated ORP and lower pH. Thus, PAW possesses a highly oxidizing (ORP and H_2O_2) and acidic nature, which may contribute to its sanitizing and antimicrobial efficacy.

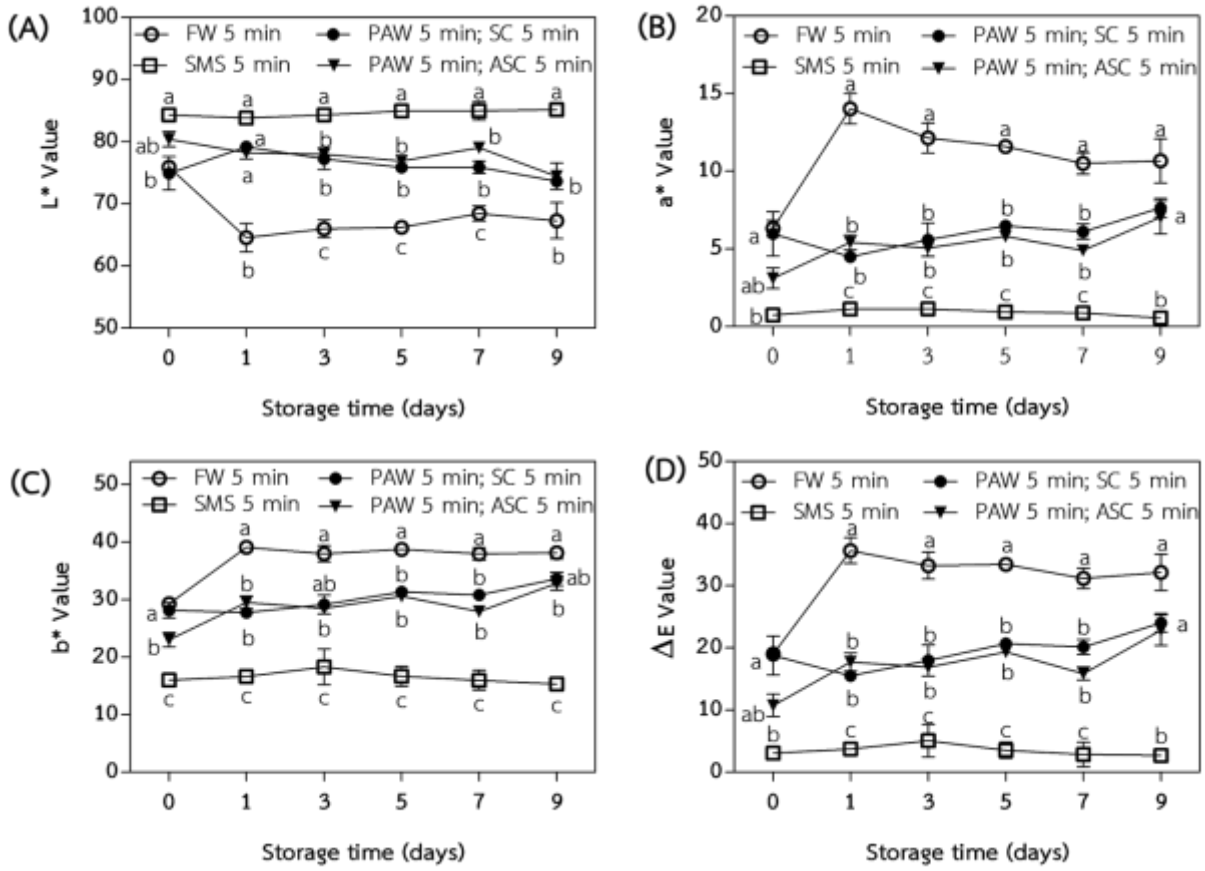

Figure 1 L* (A), a* (B), b* (C), and ΔE value (D) of aromatic coconut mesocarp treated with PAW followed by SC and treated with PAW followed by ASC compared with sodium metabisulfite (SMS) and filtered water (FW) during storage at 4°C for 9 days. The letters (a, b, and c) indicate significant differences at P<0.05. (n=12)

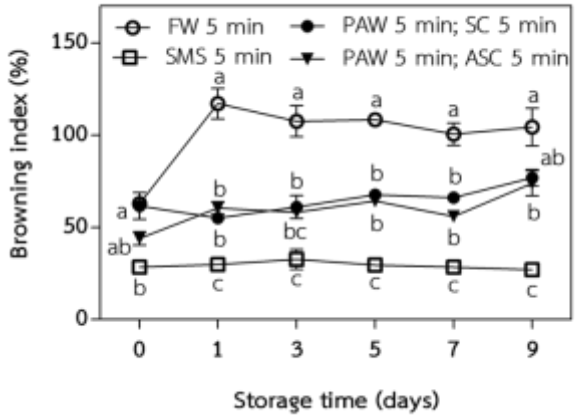

Figure 2 Browning index (BI) of aromatic coconut mesocarp treated with PAW followed by SC and treated with PAW followed by ASC compared with sodium metabisulfite (SMS) and filtered water (FW) during storage at 4°C for 9 days. The letters (a, b, and c) indicate significant differences at P<0.05. (n=12)

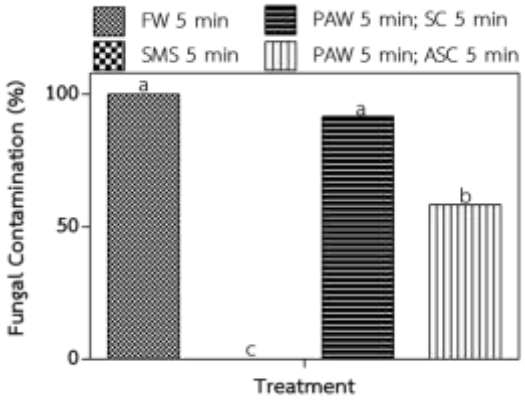

Figure 3 Fungal contamination of aromatic coconut mesocarp treated with PAW followed by SC and treated with PAW followed by ASC compared with sodium metabisulfite (SMS) and filtered water (FW) during storage at 4°C for 9 days. The letters (a, b, and c) indicate significant differences at P<0.05. (n=12)

Table 1 The pH, electrical conductivity (EC), oxidation-reduction potential (ORP), and hydrogen peroxide (H_2O_2) levels in plasma-activated water (PAW) were compared with those in filtered water and sodium metabisulfite (SMS) at 25°C (± 2°C).

Treatment	pH	EC (mS/cm)	ORP (mV)	H_2O_2 (µg/L)
Filtered water	5.76a	22.67c	219.67b	2.11b
SMS	3.86b	25,500.00a	105.00c	1.84b
PAW	2.79c	498.33b	544.33a	5.56a
F-test	**	**	**	**
C.V. (%)	1.58	0.66	1.93	12.17

The small letters (a, b, c) and the asterisk (*, **) indicate significant differences of each treatment at P<0.05.

Discussion

The current study demonstrated that the sequential application of plasma-activated water (PAW) followed by sodium chlorite (SC) or acidified sodium chlorite (ASC) at 200 ppm effectively inhibited browning in the coconut mesocarp. The anti-browning mechanism of PAW followed by SC or ASC is likely associated with the inactivation of polyphenol oxidase (PPO) by reactive species generated during PAW activation (Perinban et al., 2022). The synergistic action was further enhanced by SC, which acts as a mixed-type inhibitor of PPO activity (Lu et al., 2006), thereby preventing enzymatic browning on the surface of the coconut mesocarp. Meanwhile, ASC showed a more synergistic effect, attributable to the strong acidity of the PAW–ASC system (pH ≈ 2.3). The resulting acidic environment reduces PPO activity, as this enzyme is highly pH-dependent, thus further contributing to the inhibition of enzymatic browning.

Regarding the antifungal activity of PAW, the results showed that PAW exhibited strong acidity and oxidizing capacity, as evidenced by its low pH, high ORP, and elevated H_2O_2 levels, likely due to the formation of reactive oxygen and nitrogen species during plasma activation. Consistent with previous reports, PAW possesses low pH, high EC, and high ORP due to reactive species such as H_2O_2, NO_2^-, NO_3^-, ONOOH, and O_3 (Du et al., 2023; Fan and Wang, 2020). These reactive species can disrupt microbial cell integrity by breaking intramolecular bonds of the peptidoglycan layer through hydrogen abstraction from the peptide bonds, resulting in cell wall degradation, metabolic arrest, and cell death (Wong et al., 2023). However, PAW followed by SC showed no

significant antifungal effect, possibly due to the insufficient concentration of SC to oxidize the fungal cell wall. These findings are consistent with Payuhamaytakul et al. (2018), who reported that 250 mg/L SC did not suppress fungal growth in trimmed aromatic coconut, due to fungal cell walls containing chitin, which is more rigid than the peptidoglycan found in bacterial cell walls (Soušková et al., 2011). In contrast, PAW followed by ASC effectively inhibits fungal contamination, likely due to the low pH, which enhanced antimicrobial efficacy.

Conclusion

The combination of PAW with food additives such as sodium chlorite (SC) and acidified sodium chlorite (ASC) at 200 ppm effectively reduced browning during storage from day 1 to day 7. PAW followed by The sequential treatment of PAW followed by ASC inhibited fungal growth by up to 58%, primarily due to the synergistic effect of PAW and ASC, which exhibit low pH, high electrical conductivity (EC), and high oxidation-reduction potential (ORP). However, PAW followed by ASC could not completely replace SMS in inhibiting browning and fungal contamination. The results suggested that the PAW–chlorite sequence shows promising potential for practical application in industrial postharvest systems.

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References

Anonymous. 2000. Acidified sodium chlorite solutions. In *Code of Federal Regulations* (21 CFR 173.325). Washington, DC: Office of the Federal Register, U.S. Government Printing Office

Deadman, B. J., Hellgardt, K., and Hii, K. K. 2017. A colorimetric method for rapid and selective quantification of mixtures comprising peroxodisulfate, peroxomonosulfate, and hydrogen peroxide. *Reaction Chemistry & Engineering*, 2: 462-466. . https://doi.org/10.1039/C7RE00050B

Du, Y. H., Mi, S. N., Wang, H. H., Yang, F. W., Yu, H., Xie, Y. F., and Yao, W. R. 2023. Inactivation mechanism of *Alternaria alternata* by dielectric barrier discharge plasma and its quality control on fresh wolfberries. *Food Control*, 148, Article 109620. https://doi.org/10.1016/j.foodcont.2023.109620

Fan, X., and Wang, W. 2020. Quality of fresh and fresh-cut produce impacted by nonthermal physical technologies intended to enhance microbial safety. *Critical Reviews in Food Science and Nutrition*, 62(2), 362–382. https://doi.org/10.1080/10408398.2020.1816892

Friedman, M. 1996. Food browning and its prevention: An overview. *Journal of Agricultural and Food Chemistry*, 44(3), 631–653. https://doi.org/10.1021/jf950394r

Lien, K. W., Hsieh, D. P. H., Huang, H. Y., Wu, C. H., Ni, S. P., and Ling, M. P. 2016. Food safety risk assessment for estimating dietary intake of sulfites in the Taiwanese population. *Toxicology Reports*, 3, 544–551. https://doi.org/10.1016/j.toxrep.2016.06.003

Lopez, A. S. 2023. A concise review on tender and mature coconut water: Its application in food. *The Pharma Innovation*, 12(8), 835–848. https://www.thepharmajournal.com/archives/2023/vol12issue8/PartJ/12-7-452-793.pdf

Lu, S., Luo, Y., and Feng, H. 2006. Inhibition of apple polyphenol oxidase activity by sodium chlorite. *Journal of Agriculture Food Chemistry*,54, 3693-3696. https://doi.org/10.1021/jf0518103

Luo, Y., Lu, S., Zhou, B., and Feng, H. 2011. Dual effectiveness of sodium chlorite for enzymatic browning inhibition and microbial inactivation on fresh cut apples. *LWT- Food Science and Technology*, 44 (7), 1621-1625. https://doi.org/10.1016/j.lwt.2011.02.015

Payuhamaytakul, K., Jitareerat, P., Uthairatanakij, A., Srilaong, V., and Remunrani, P. 2018. Effectiveness of sodium chlorite and acidified sodium chlorite to inhibit mesocarp browning of trimmed aromatic coconut. *Acta Horticulturae*, 1306, 179-186. https://doi.org/10.17660/ActaHortic.2018.1210.11

Perinban, S., Orsat, V., and Raghavan, V. 2022. Influence of plasma activated water treatment on enzyme activity and quality of fresh-cut apples. *Food Chemistry, 393*, 133421. https://doi.org/10.1016/j.foodchem.2022.133421

Soušková, H., Scholtz, V., Julák, J., Kommová, L., Savická, D., and Pazlarová, J. 2011. The survival of micromycetes and yeasts under the low-temperature plasma generated in electrical discharge. *Folia Microbiologica*, 56(1), 77–79. https://doi.org/10.1007/s12223-011-0005-5

Treesuwan, K., Jirapakkul, W., Tongchitpakdee, S., Chonhenchob, V., Mahakarnchanakul, W., Moonmangmee, S., and Tongkhao, K. 2022. Effect of controlled atmospheric conditions combined with salt acid immersion on trimmed young coconut qualities during cold storage. *Food Packaging and Shelf Life, 32*, 100857. https://doi.org/10.1016/j.fpsl.2022.100857

U.S. Food and Drug Administration. 1998. *Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* (pp. 50–200 ppm total chlorine recommendation). https://www.fda.gov/media/117408/download

Walker, J. R. L., and Wilson, E. L. 1975. Studies on the enzymatic browning of apples: Inhibition of apple o-diphenol oxidase by phenolic acids. *Journal of the Science of Food and Agriculture*, 26(12), 1825–1831. https://doi.org/10.1002/jsfa.2740261204

Wong, K.S., Chew, N.S.L., Low, M., and Tan, M.K. 2023. Plasma-activated water: physicochemical properties. *Generation Techniques and Applications Processes*, 11, 2213. https://doi.org/10.3390/pr11072213