



Research article

Alternative postharvest pre-treatment strategies for quality and microbial safety of 'Granny Smith' apple



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ABSTRACT

Over the years, chemical pre-treatments have been used intensively to maintain apple quality and reduce decay during postharvest. This conduct has been reported to have a negative impact on environment and human health. This study aimed to investigate alternative approaches such as hot water (HW) and electrolyzed water (WE) treatments for decay management of 'Granny Smith' apples. Two different sets of experiments were set up for this study. In experiment 1, the effects of HW treatment (45 °C) under varying dipping durations (5, 10 and 15 min) on physicochemical quality of apple were investigated. In experiment 2, the curative efficacy of slightly alkaline electrolyzed water (SAI-EW) (50, 100, 200, 300, 400 and 500 mg L⁻¹) against *Botrytis cinerea* was investigated. Hot water treatment duration (15 min) had beneficial effects on flesh firmness, fruit colour, total soluble solid (TSS) and titratable acidity (TA) by the end of the storage. In contrast, a significant reduction in fruit weight and TA values ($p < 0.05$) were observed in control fruit. The SAI-EW treatments against *B. cinerea* resulted in a significant reduction in lesion zones compared to the untreated control fruit. Curative efficacy was most effective at concentrations of 200–500 mg L⁻¹ for 5 °C and 300–500 mg L⁻¹ for 24 °C. These findings suggest the potential of combining lower concentrations of SAI-EW with other hurdle techniques for better preservation of fresh apples.

1. Introduction

Apples (*Malus domestica*) fruit are commonly stored for long duration at low temperatures under normal or controlled atmosphere conditions. In this period, apple fruit gradually loses its qualities mostly related to enzymatic or oxidative and metabolic changes as well as fungal diseases. The postharvest loss of apple fruit is globally ranging from 20 to 50% in developing countries (Kitinoja and Kader, 2015) and 25–50% in South Africa (Den Breeyen et al., 2020; Louw, 2016; Wand et al., 2006). Most importantly, apples are susceptible to a high degree of fungal pathogens including, *Botrytis cinerea*, which is the main cause for the development of black, grey and blue mould (Moschetti et al., 2013). Therefore, the application of various pre-treatments before long-term storage has been the common practice in pack-houses to control chilling injury, browning and decay. For the past decades, chemical pre-treatments such as sodium hypochlorite (chlorine), calcium chloride or diphenylamine (DPA), hydrogen sulfide have been applied to maintain the quality of apple fruit

during storage (Ramirez et al., 2019). However, there is a great public health concern due to the presence of chemical residues on the fruit and in the environment. This has motivated researchers to develop alternative pre-treatment strategies (Fenik et al., 2011). This include thermal or non-thermal treatments such as, hot water and electrolyzed water, which have emerged as a safe and effective techniques (Ferri et al., 2016; Hung et al., 2010; Usall et al., 2016).

Studies had shown the positive effects of hot water (HW) treatments for better shelf life of apple during postharvest storage. On the study done for 'Ingrid Marie' apple, Maxin et al. (2004) applied HW treatment at 49 °C, 51 °C and 53 °C for 1, 2 and 3 min and stored under normal air condition at 2 °C for 4 months. Similarly, the effects of HW treatment has been reported for various apple fruit cultivars such as, 'Elstar' apple using 50–52 °C for 2 min and 54–56 °C for 3 min (Maxin, 2012), 'Topaz' apple using 51 °C for 2 min (Neuwald and Kitemann, 2016), 'Red Delicious' apple using 45 °C for 10 min (López-López et al., 2013). Based on the findings from these studies, HW treatment did not have a significant effect on 'Elstar' apple

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firmness and sugar content, but colour parameters (b^* and a^*) increased significantly at higher temperature treatment (Maxin, 2012). Similarly, HW had no significant influence the firmness, TSS and TA of 'Topaz' apples (Neuwald and Kitemann, 2016). On the other hand, HW treatment significantly increased a^* value and decreased the firmness of 'Red Delicious' apple (López-López et al., 2013). The outcomes of these studies demonstrated that the effect of HW treatments varies according to the temperature of the hot water, treatments duration and cultivar.

Electrolyzed water (EW) treatment is an emerging hurdle technique, which is cost effective and environmentally friendly (Huang et al., 2006). It is also has a strong bactericidal effect and has been shown to be a scavenger of free radicals (Kim and Hung, 2014). Post-harvest application of EW has been shown to successfully control microbial contaminations in various circumstances on citrus, strawberries, pears, and fresh-cut vegetables (Al-Haq et al., 2002; Fallanaj et al., 2016; Qi et al., 2018). For instance, Qi et al. (2018) demonstrated effective removal of pesticide residues from spinach, snap beans and grapes without affecting their colour and texture. Ferri et al. (2016) reported on the EW treatment (400 mg L⁻¹) of 'Cripps Pink' apple fruit that was stored at 1 °C for 4 months. The authors observed improved storage quality and shelf life as well as a significant reduction the concentration of some pesticide residues on the fruit.

Grey mould caused by *Botrytis cinerea* is currently the major pathogen that causes post-harvest loss of apples (Mbili et al., 2017). However, there is no report related to the application of EW to control the growth of *Botrytis cinerea* in 'Granny Smith' apple fruit. Despite the highest demand of 'Granny Smith' apple due to its antioxidant properties and its susceptibility to quality loss during postharvest handling and storage, there has not been much done to apply physical pre-treatments using HW or EW. Therefore, the following hypotheses were to be tested: (i) that lowering the hot water temperature and extending the dipping during would not impact the fruit quality; (ii) that the least slightly alkaline electrolyzed water (SAL-EW) concentration selected will have inhibitory effect against *B. cinerea* *in vivo*. Thus, this study carried out two separate experiments. In the first experiment, the impacts of HW treatment at 45 °C and dipping duration (5, 10, and 15 min), on physicochemical quality of 'Granny Smith' apple stored at 10 °C for 21 days was investigated. In the second experiment, the curative efficacy of slightly alkaline electrolyzed water (SAL-EW) treatment (with pH 10-11 and oxidation-reduction potential (ORP, > -800 mV) against *B. cinerea* on 'Granny Smith' apple stored at 5 °C for 21 days was investigated.

2. Materials and methods

2.1. Fruit

In this study, two consecutive experiments were conducted. 'Granny Smith' apple fruit were obtained at commercial maturity from fresh fruit retail market, Stellenbosch, Western Cape, South Africa, and transported in cooled conditions to the Agricultural Research Council (ARC) - Infruitec-Nietvoorbij, Stellenbosch, South Africa. Only mature, healthy and unblemished fruit were selected, and once sorted the apples were surface disinfected with 70% ethanol (v/v) and allowed to air dry and stored at 0 °C prior to treatments.

2.2. Experiment I

2.2.1. Hot water treatment

For hot water treatment, apples were divided into four batches of 36 fruit each: three of these batches were exposed to hot water (45 °C), each batch for a dipping duration of 5, 10 and 15 min, respectively, using Brookfield TC500 water bath. The fourth batch was stored without HW treatment as a control. After treatments, fruit were air dried in the laboratory and stored at ≈ 10 °C with 95% relative humidity (RH) for 21 days. Samples from each treated batch and the control were taken out on day 0, 7, 14 and 21, for physicochemical quality analysis.

2.2.2. Core temperature

In order to establish the extent to which the hot water heat penetrated the internal pulp temperature of the treated fruit were measured. Samples were taken immediately after the HW dipping durations and the internal temperature was measured by inserting a thermosensor (TFX410 Ebro, Xylem Analytics, Germany) into the core of the fruit for about 60 s for a stable reading.

2.2.3. Weight loss and tissue strength

The percentage weight (%) of HW treated and control apple was measured in gram (g) using a balance scale linked to the texture analyser (FTA20, Güss, South Africa). Weight loss was then calculate using Eq. (1). The tissue strength (hardness) of each fruit sample was measured using a texture analyser (FTA20, Güss, South Africa). The two opposite sides (left, right) of the fruit were gently peeled, placed on the platform and a 7.9 mm compression probe was used on each of the side with a penetration distance of 8.9 mm and a speed of 10 mm s⁻¹; the results were expressed in Newton (N). The weight loss and texture analysis were done in triplicate using ten fruit in each replica.

$$WL = \frac{W_o - W_f}{W_o} \times 100 \quad (1)$$

where, WL is the weight loss (%); W_o and W_f are the initial weight (g) and the final weight (g) of the apples, respectively.

2.2.4. Surface colour

Colour changes on each apple fruit was measured based on Commission International del' Eclairage (CIE) colour system using a digital Chroma-meter (CR 400/410 Konica Minolta Sensing Inc., Japan). Colour calibration of the chroma-meter was performed against a white and black tile background prior to each measurement. Colour measurements were taken using individual fruit ($n = 3$) and data obtained were average of individual colour parameters To describe the measured colour attributes hue angle (h°), which describes the qualitative attribute of colour shades (0° = red-purple and 180° = bluish-green), and Chroma (C^*), which denotes the quantitative attribute of colour intensity were calculated (Caleb et al., 2016) using Eqs. (2) and (3):

$$h^\circ = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (2)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

where, L^* denotes the lightness, a^* describes red (+)/green (-) and b^* for yellow (+)/blue (-).

2.2.5. Total soluble solid (TSS) and titratable acidity (TA)

Fruit were processed in to juice using a juice extractor (4294 J700, Braun, China) and the juice obtained was used to measure total soluble solid (TSS) and titratable acidity (TA). Total soluble solid was measured using a pocket calibrated refractometer (PAL-1, ATAGO, and Japan) and the results were expressed as °Brix. The titratable acidity of each fruit were obtained from the titration of 53.7 mL of each fruit juice with 0.333 N of sodium hydroxide (NaOH) at a pH of 8.2, using Crison Titromatic 1S/2B (Crison Instruments, Barcelona, Spain) and the results were expressed as g 100 mL⁻¹.

2.3. Experiment II

2.3.1. Pathogen isolation

Fungal pathogen *B. cinerea* (Accession number: PPRI 7338) obtained from the Agricultural Research Council - Plant Protection Institute, Pretoria, South Africa. *B. cinerea* was cultured on potato dextrose agar (PDA, pH 5.6, Merck, and Johannesburg, South Africa) at 25 °C for 3 days for mycelial plugs and 7 days for the production of spores. The cultures of

B. cinerea were maintained on PDA slants at 4 °C. Conidia were harvested from the medium surface with sterile distilled water together with Tween 80 (0.05% W/V), and gently agitating the plates to dislodge the spores. The final inoculum concentration was adjusted to 1×10^4 conidia mL⁻¹ using a haemocytometer (Superior, Germany).

2.3.2. Electrolyzed water preparation

In this study, the electrolyzed water generator was developed and assembled by ECA Technology Africa (Cape Town, South Africa) consisting of a water tank, electrolytic cell, power supply, and master flex according to the principal of SAI-EW generation. The SAI-EW was generated by electrolysis of a combined solution of dilute hydrochloric acid in the range 0.001–0.9% (ECA Tech Africa, SA) and sodium chloride as electrolyte. The electrolyte flow passed through an electrolytic cell at a rate of 2 mL min⁻¹, at setting of current 3.8–3.9 V, amperage 10 A. The electrolyte was diluted with micro-filtered tap water at a flow rate of 4 L min⁻¹ using a pump to generate SAI-EW. The pH and oxidation reduction potential were measured immediately after production and right before experiments to confirm that they were not changed. The measured available chlorine concentration (ACC) in the SAI-EW was 500 ppm, the oxidation reduction potential (ORP) was > -800 mV. This was measured with an ORP meter (HM-60V, TOA Electronics Ltd., Tokyo, Japan), and pH was ≈11, which was measured using a pH meter (D-22, Horiba, Kyoto, Japan).

2.3.3. Fruit treatment and storage

Apple fruit were randomly divided into homogenous groups of twenty-one batches (box with 150 apples) representing the seven treatments and three levels (Table 1). A cork-borer (3 mm × 3 mm) was used to uniformly wound the ‘Granny Smith’ apples on opposite ends, and then inoculated with a spore concentration of 1×10^4 spores L⁻¹ of *B. cinerea* according to the protocol from Postharvest Pathology Laboratory, Agricultural Research Council, (ARC) Infruitec-Nietvoorbij South Africa. Inoculated fruit was enclosed with black plastic bags with a wet paper towel to ensure high humidity and promote spore germination for 20 h at 20 °C. After inoculation, the samples were allowed to air-dry for ≈6 h. Thereafter, the apples per batch were dipped in SAI-EW with varying concentrations of ACC (50, 100, 200, 300, 400 and 500 mg L⁻¹) for different time intervals (5 min, 10 min and 15 min). Measurements of the inoculated/lesion zones (lesion length (mm), area (mm) and colour) were carried out weekly at 5 °C, and after two days of storage at 24 °C for shelf-life study. A minimum of three replicate for each batch per treatment was taken on the sampling day (1, 7, 14, and 21) for image analysis.

2.3.4. Visual quality and image analysis

Visual quality assessment was done to determine the effect of electrolyzed water treatment on the lenticle appearance of apple fruit. The efficacy of EWT on the inhibition of *B. cinerea* was investigated via image analysis using Nikon E100 NIS Elements imaging software fitted with a Siedentop Trinocular Tube (Basic Research version 3.10 Inc., Nikon

Instruments Europe B.V., AS Amstelveen, The Netherlands). For analysis, digital image (2048 × 1536 pixels) of ‘Granny Smith’ apple was taken using Canon 650D DSLR camera fitted with an 18-megapixel sensor. Six images were captured for each treatment concentration and dipping duration. For each image, the area (surface area of the image in μm² for the lesion zone) were measured automatically. Images were measured after the micrometer scale was calibrated to pixel size using the program's calibration function (1 pixel = 0.16 μm at 400 × magnification). The saturation tool was used to measure the colour of the apple. In addition, white saturation intensity was adjusted to ensure optimal contrast of the image with the background. The resulted lesion zone was expressed in mm.

2.3. Statistical analysis

To elucidate on the impacts hot water treatment dipping duration on physico-chemical qualities and electrolyzed water concentration, treatment time and storage duration against *Botrytis cinerea*, factorial ANOVA was applied at 95% confident interval using Statistica Software (version 13, StatSoft Inc. TIBCO Software Inc., USA). Post-hoc test (Duncan multi range test) was also used to determine the difference between mean combination values. Results were presented as mean value ($n = 3$) ± standard deviation.

3. Results and discussions

3.1. Experiment I

3.1.1. Fruit core temperature and heat transfer

The fruit core temperature of apple fruit after HW treatment was significantly lower than the surface temperature. However, the highest core temperature (34.13 ± 0.21 °C) for treated samples was observed for fruit dipped for 15 min, while the lowest was for samples dipped for 5 min (20.07 ± 0.25 °C). The internal temperature for non-treated control samples was relatively close to that of the initial holding storage temperature (16.67 ± 0.00 °C), as shown in Figure 1A. Similar observation to this finding was reported by Kabelitz et al. (2019), where apple treated at 55 °C for 0.5 min only affected the surface outer cell layer, while after 1 min the thickness of the heated tissue early doubled. The study further reported that the maximum temperature of apple peel tissue did not exceed more than 35 °C even the treatment duration is extended to 5 min.

Furthermore, the internal temperature of the treated and the control fruit continued to decline with the extension of the storage duration as presented in Figure 1B. No statistically significant differences were observed in core temperature from day 7. At the end of the storage, the highest internal temperature (13 ± 0.00 °C) was observed for HW-T3 samples followed by HW-T2 (12.67 ± 0.58 °C), while HW-T1 and HW-C samples had the lowest similar internal temperature (12 ± 0.0 °C). It noteworthy from this study that internal temperature of the fruit samples were slightly higher than the storage/ surrounding temperature. This could be attributed to the internal heat generated as a result of respiration process “respiratory heat” (Kabelitz et al., 2019; Wang et al., 2001).

3.1.2. Weight loss

The effects of HWT on maintaining the weight loss of treated and control apples were significantly ($P < 0.05$) different. Comparing the weight loss of the treated and un-treated fruit, the control fruit lost significant weight throughout the storage duration and reached 20% (208 ± 5.29 g) at the end of day 21. By the end of the storage a slight increase in weight was observed for all HW treated fruit as at 45 °C increased from the initial value of 239.00 ± 25.70 g to 259.67 ± 11.93 g, 269.67 ± 14.15 g and 269.33 ± 11.71 g for 5, 10 and 15 min treatment durations, respectively but there was no significant difference (Table 2). Similar to the treated fruit, no significant change was observed on the weight loss of control fruit after 14 days of storage. Nevertheless, the weight loss was found higher for the control fruit than on all HWT

Table 1. Sample name hot water treatment duration and of electrolyzed water concentration used to treat ‘Granny Smith’ apple fruit stored for 21 days at 10 °C.

Experiment I		Experiment II	
Sample names	Hot water (45 °C) dipping duration (min)	Sample name(s)	Electrolyzed water _ NaCl concentration (mg L ⁻¹)
HW-T1	5	EW-1	50
HW-T2	10	EW-2	100
HW-T3	15	EW-3	200
HW-C	0	EW-4	300
		EW-5	400
		EW-6	500
		Control (C)	0

EW, is electrolyzed water, HT, is hot water, T is treatment.

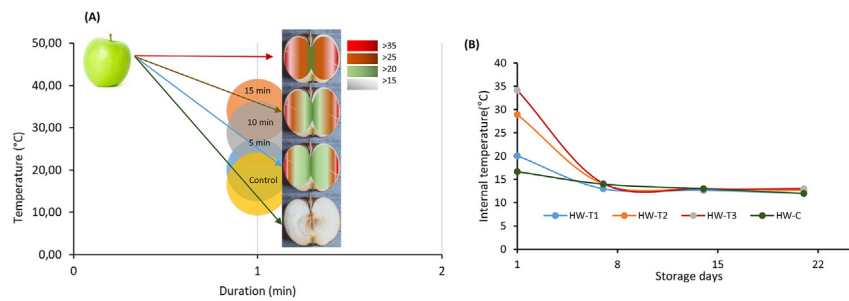


Figure 1. Effects of hot water treatment (HWT) at 45 °C on the internal temperature of ‘Granny smith’ apple fruit treated for 5 min (HW-T1), 10min (HW-T2) and 15min (HW-T3) and control (HW-C), surface and core temperature, measurement after 1 min (A) and during storage at 10 °C for 21 days (B).

Table 2. Effects of hot water treatment (HWT) durations (5, 10 and 15 min) on the instrumental physical qualities of ‘Granny Smith’ apple stored for 21 days.

Parameter	Storage days	Treatments			
		HW-T1	HW-T2	HW-T3	HW-C
Weight loss (g)	0	239.00 ± 25.71 ^b	239.00 ± 25.71 ^b	239.00 ± 25.71 ^b	239.00 ± 25.71 ^a
	7	269.00 ± 4.58 ^{ab}	277.33 ± 6.11 ^a	278.00 ± 4.56 ^a	203.67 ± 5.04 ^b
	14	263.00 ± 29.82 ^{ab}	269.00 ± 7.21 ^a	259.67 ± 3.21 ^b	191.67 ± 11.93 ^b
	21	259.00 ± 11.91 ^a	269.670 ± 14.15 ^a	269.33 ± 11.72 ^a	208.00 ± 5.71 ^b
Tissue strength (kg)	0	80.81 ± 0.82 ^a	80.81 ± 0.82 ^a	80.81 ± 0.82 ^a	80.81 ± 0.82 ^a
	7	83.56 ± 0.92 ^a	68.45 ± 0.35 ^c	68.55 ± 0.35 ^c	71.78 ± 0.46 ^{bc}
	14	78.06 ± 0.68 ^{ab}	66.19 ± 0.22 ^c	69.14 ± 0.39 ^{bc}	74.14 ± 0.27 ^a
	21	69.23 ± 0.63 ^b	68.25 ± 0.32 ^b	79.82 ± 0.32 ^a	81.20 ± 0.26 ^a
Colour					
<i>L</i> *	0	64.38 ± 1.79 ^a	64.38 ± 1.79 ^a	64.38 ± 1.79 ^a	64.38 ± 1.79 ^a
	7	64.95 ± 3.53 ^{ab}	62.61 ± 1.62 ^b	60.99 ± 2.26 ^b	66.86 ± 0.24 ^a
	14	64.45 ± 2.30 ^b	61.22 ± 2.07 ^c	60.53 ± 0.68 ^c	67.88 ± 2.67 ^a
	21	65.87 ± 0.76 ^b	61.36 ± 0.44 ^c	66.32 ± 1.51 ^{ab}	66.96 ± 2.62 ^{ab}
<i>a</i> *	0	-17.86 ± 2.27 ^a	-17.86 ± 2.27 ^a	-17.86 ± 2.27 ^a	-17.86 ± 2.27 ^a
	7	-18.52 ± 1.04 ^b	-18.72 ± 0.09 ^b	-19.63 ± 1.02 ^{ab}	-17.97 ± 1.82 ^b
	14	-18.05 ± 1.11 ^a	-18.82 ± 1.31 ^a	-18.82 ± 1.08 ^a	-17.90 ± 2.42 ^a
	21	-18.07 ± 0.51 ^a	-17.49 ± 0.62 ^a	-17.49 ± 0.45 ^a	-17.95 ± 0.55 ^{ab}
<i>h</i> °	0	118.81 ± 1.66 ^a	118.81 ± 1.66 ^a	118.81 ± 1.66 ^a	118.81 ± 1.66 ^a
	7	113.85 ± 1.61 ^a	113.72 ± 0.58 ^a	113.94 ± 0.86 ^a	113.14 ± 0.29 ^a
	14	113.61 ± 1.03 ^a	113.91 ± 1.16 ^a	113.39 ± 0.52 ^a	111.43 ± 1.89 ^a
	21	113.09 ± 0.68 ^a	113.67 ± 0.80 ^a	111.43 ± 1.53 ^b	111.21 ± 0.84 ^b
<i>C</i> *	0	47.84 ± 3.03 ^a	47.84 ± 3.03 ^a	47.84 ± 3.03 ^a	47.84 ± 3.03 ^a
	7	45.86 ± 1.87 ^a	46.59 ± 0.85 ^a	46.48 ± 2.21 ^a	47.44 ± 2.26 ^a
	14	45.19 ± 4.48 ^a	46.04 ± 2.01 ^a	47.34 ± 1.79 ^a	48.86 ± 2.80 ^a
	21	46.10 ± 2.21 ^b	48.63 ± 0.32 ^a	47.55 ± 4.03 ^a	49.64 ± 0.55 ^a

Mean (n = 3) ± standard deviation in the same columns with different low cases superscripts indicates significant difference (p < 0.05) along the storage duration. HT = hot water, T1, T2, T3, are Treatment 1, 2, 3 respectively and C is control.

‘Granny Smith’ apple fruit at the end of the storage (Table 2). These results are contradicting with the result reported by Moschetti et al. (2013) where, significant weight loss with the average of 11.12 ± 0.23 g was found on Golden Delicious’ apple dipped at 45 °C for 10 min and stored for 50 days at 2 °C with 90% RH. For ‘Gala’ apple treated at 38 °C for 4 days and stored for 8 weeks at 0 °C, a progressive weight loss during storage was also observed. This study associated the weight loss of ‘Gala’ apple with the increase in respiration rate of the fruit due to heat treatment and inappropriate application of HT, since the efficacy of HT is dependent on cultivar, heat damage tolerance, and handling procedure. Therefore, the observed differences on the above-mentioned studies could be due to the difference in cultivar, storage duration and treatment and storage temperature. The differences in microstructure and cell adhesion properties between apple fruit cultivars which influences the moisture movement then leads to weight loss were reported by Ng et al. (2013).

3.1.3. Tissue strength

As shown in Table 2, the tissue strength of HWT and control apple fruit was significantly (P < 0.05) different after day 7 of storage. Hot water treatments for 15 min (HW-T3) and control (HW-C) fruit maintained highest tissue strength until the end of the storage duration. On the other hand, the gradual decrease of firmness during storage was observed for apple treated for 5 and 10 min from 80.81 ± 0.82 N to 69.23 ± 0.63 N and 68.25 ± 0.32 N, respectively, by the end of the storage but a significant reduction was observed only at 10 min treatment. On the other hand, apple fruit firmness was maintained from 80.81 ± 0.82 N to 79.82 ± 0.32 N and 81.20 ± 0.26 N under HW-T3 and HW-C at day 21, respectively. Similarly, Moschetti et al. (2013) reported that HWT (45 °C for 10 min) and control ‘Golden Delicious’ apple fruits were firmer. According to this study, exposure to a non-tolerated high temperature for an excessive time could cause higher or no significant loss of firmness compared to control fruit.

Table 3. Effects of hot water treatment (HWT) durations (5, 10 and 15 min) on the TSS and TA values of ‘Granny Smith’ apple stored for 21 days.

Parameter	Storage days	Treatments			
		HW-T1	HW-T2	HW-T3	HW-C
TSS (°Brix)	0	13.00 ± 0.00 ^a	13.00 ± 0.00 ^a	13.00 ± 0.00 ^a	13.00 ± 0.00 ^a
	7	12.63 ± 0.46 ^b	12.77 ± 0.05 ^b	12.50 ± 0.00 ^b	13.93 ± 0.05 ^a
	14	13.23 ± 0.05 ^b	13.20 ± 0.10 ^b	12.63 ± 0.23 ^c	14.20 ± 0.10 ^a
	21	12.67 ± 0.05 ^b	12.67 ± 0.05 ^b	13.40 ± 0.17 ^a	13.53 ± 0.05 ^a
TA (malic acid, g 100 mL ⁻¹)	0	1.01 ± 0.00 ^a	1.01 ± 0.00 ^a	1.01 ± 0.00 ^a	1.01 ± 0.00 ^a
	7	1.24 ± 0.03 ^a	1.02 ± 0.02 ^b	1.20 ± 0.06 ^a	1.05 ± 0.03 ^b
	14	1.35 ± 0.02 ^a	1.04 ± 0.02 ^c	1.15 ± 0.06 ^b	1.03 ± 0.02 ^c
	21	1.40 ± 0.12 ^a	1.03 ± 0.00 ^b	1.03 ± 0.02 ^b	0.85 ± 0.00 ^c

Mean (n = 3) ± standard deviation in the same columns with different low cases superscripts indicates significant difference (p < 0.05) along the storage duration. HT = hot water, T1, T2, T3, are Treatments for 5, 10, and 15 min dipping duration, respectively, and C is control.

Comparing the values during the storage durations, it was observed that fruit treated at 45 °C for 5 min (HW-T1) has the highest firmness value (83.56 ± 0.92 N) at day 7 while, fruit treated at 45 °C for 10 min (HW-T2) had the lowest firmness value of 68.19 ± 0.225 N at day 14. These could be due to the shorter treatment duration and storage time, which would retain decrease in the viscosity of soluble pectin as, expected from prolonged storage at low temperature results the increase in viscosity of soluble pectin. The mechanism of delayed softening could be linked to a decrease in the activity of cell wall hydrolytic enzyme and an increase in calcium bonded to the cell wall. Li et al. (2013) reported loss of firmness in heat treated ‘Red Fuji’ apples at 45 °C and 60 °C after 28 days of storage. A decrease in firmness for heat treated (38 °C) apples (cv. Gala) for 4 days and stored at 0 °C for 8 weeks was reported by Shao et al. (2012). According to Rab et al. (2012), the lowest firmness found on hot water treated fruit could be due to the activity of the softening-related enzyme.

3.1.4. Surface colour

As presented in Table 2, the HWT duration had a significant effect on colour parameters (L^* , a^* and b^*) during storage. The results showed that, the highest L^* value was observed for 15 min HW treated apple (HW-T3) with the increase of 66.3 ± 1.52 by the end of the storage from 64.4 ± 1.79 initial value. On the other hand, HWT of apple for 5 min (HW-T1) maintained the initial surface colour of fruit throughout the storage duration. However, a significantly highest L^* value was observed for HW-C fruit with an initial value of 64.4 ± 1.79, increased to 66.9 ± 2.62 by the end of the storage. The observed highest L^* value under HW-T3 and control fruit could be an indication for loss of the green fruit colour. López-López et al. (2013) also reported a decreased in the lightness of apple (*Malus domestica* Borkh) treated at 45 °C for 10 min followed by a cool in a 21 °C water for 5 min.

On the other hand, the effect of HWT on the a^* value of apple was not significant during the storage duration and the values were similar with the control fruit. Since a^* value indicated the change in colour from green (negative values) to red (positive values), the results indicated the colour change from yellow to blue, maintenance of the greenness a^* value of treated fruit. While loss of green colour drove to the fast browning of control fruit and may be due to the browning-involve enzyme (Barrett et al., 2010). A slight decreased in h^* values were observed for all treated and control fruit during storage. The h^* reduced from 118.81 ± 1.66 at day zero to 113.09 ± 0.68, 113.67 ± 0.80, 111.43 ± 1.53 and 111.21 ± 0.84 for HW-T1, HW-T2, HW-T3 and HW-TC, respectively. A significant reduction of C^* value was observed for HW-T1 from 47.84 ± 3.03 initially to 46.10 ± 2.21 by the end of the storage day 21 compared to other samples. The C^* value increased to 48.63 ± 0.32 for HW-T2 and maintained by HW-T3 but a significant increment was observed for HW-C, where the value reached to 49.64 ± 4.03 by the end of the storage. During storage the slight decrease in C^* values associated with the loss of colour intensity (Palma et al., 2015),

whereas, the higher and lower value of h^* can be associated with fresh green and yellowish fruit colour, respectively (Bessemans et al., 2016). Therefore the higher C^* and h^* values observed in HW-T2 could give an indication that the fruit colour attributes were maintained at this treatment condition. However the effects of the treatments showed no significant difference in both h^* and C^* values except day 21. The decrease in h^* and C^* values were reported by Moschetti et al. (2013) for ‘Golden Delicious’ and Wang et al. (2006) for ‘Red Delicious’ apple due to heat treatment. According to Li et al. (2013), the evolution of colour parameters related to heat treatment mainly depends on the temperature and the duration of exposure. The current study clearly demonstrated that the different in colour lightness due to the exposure time of the fruit to the HWT. Whereas, the difference in h^* and C^* indicated the effects of treatment duration.

3.1.5. Total soluble solid (TSS) and titratable acidity (TA)

As demonstrated in Table 3, the TSS value of ‘Granny Smith’ apple significantly increased from 13.00 ± 0.00 to 13.4 ± 0.17 and 13.50 ± 0.05 under HW-T3 (15 min) and HW-C (control), respectively by day 21. On the contrary, the TSS value decreased from 13 ± 0.00 from day 0–12.67 ± 0.05 for both apple fruit treated for 5 (HW-T2) and 10 min (HW-T2) at 45 °C by the end of the storage (21 days). Comparing both treated and control fruit, the highest increase in TSS value was observed under control fruit. Hemmaty et al. (2007) also reported lower TSS value of ‘Golden Delicious’ apple treated at 25 °C for 10 min, 38 °C for 5 min, 54 °C for 1 min and kept at 1 °C ± 16 months plus 7 days in 25 °C. According to Mahajan (1994) TSS, generally increased during storage due attributed to the breakdown of starch into sugars. Furthermore, since TSS percentage is a function of moisture content and the total dissolved solids of the fruit, thus lower TSS in fruit treated with hot water may be assigned to the low moisture loss (Díaz-Pérez et al., 2001).

Contrary to the slight increase in TSS value, the titratable acidity (TA) expressed as malic acid significantly (p ≤ 0.05) decreased from 1.01 ± 0.00 g 100 mL⁻¹ to 0.85 ± 0.00 g 100 mL⁻¹ for control fruit at the end of storage duration (Table 3). However, the effect of HW treatment increased of TA for all treated samples from 1.01 ± 0.00 g 100 mL⁻¹ to 1.40 ± 0.12 g 100 mL⁻¹, 1.03 ± 0.00 g 100 mL⁻¹, and 1.03 ± 0.02 g 100 mL⁻¹ for HW-T1, HW-T2 and HW-T3, respectively. According to Cherian et al. (2014), some fruit could use malic acid as a substrate for the respiration process, which leads to a decrease in TA value during cold storage. Therefore, the highest TA values for apple treated for 5 min and the lowest under control fruit could be associated with inhibition effect of HWT which leads to lower respiration under 5 min treated fruit. Li et al. (2013) reported a decrease in TA value of heat-treated ‘Red Fuji’ and ‘Golden Delicious’ at 45 °C for 3 h after 28 days of storage at 0 °C, however, the authors noted that heat treatment had not effect on TSS value of both cultivars. Similarly, Shao et al. (2012) demonstrated the decline of TA and an increase in TSS value for heat treated (38 °C) apple (cv. Gala) for 4 days and stored at 0 °C for 8 weeks.

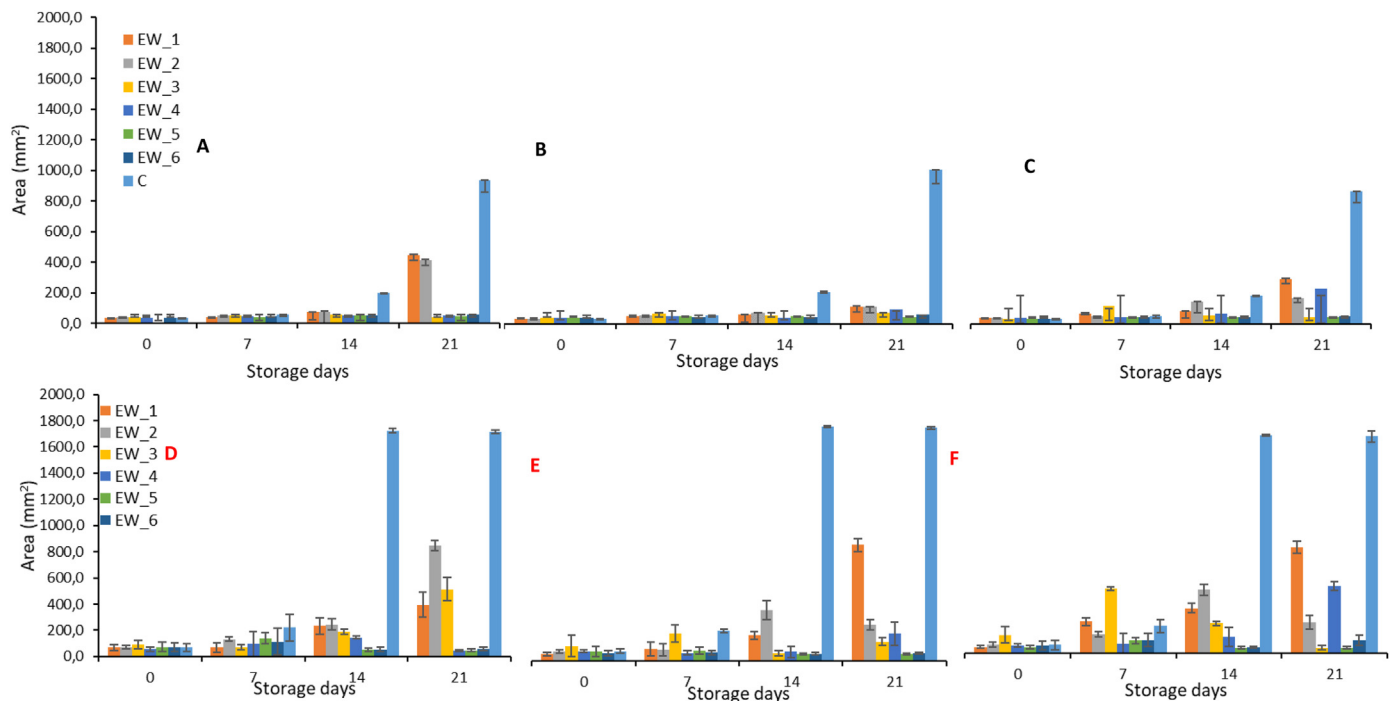


Figure 2. Effect of SAI-EW concentrations (Table 1), treatment time (5 min (A), 10 min (B) and 15 min (C)) at 5 °C and (5 min (D), 10 min (E) and 15 min (F)) at 24 °C and storage duration on the growth area of *B. cinerea* on 'Granny Smith' apple stored.

3.2. Experiment II

3.2.1. Visual quality

The visual quality analysis was carried out to identify observe any possible lenticel damage due to the SIA-EW treatments on 'Granny Smith' apples. The results obtained showed that the SIA-EW at higher concentration affected the lenticel colour appearance during storage in comparison to the control samples. The highest EW concentrations (400 and 500 mg L⁻¹) negatively affected the lenticels of apple fruit and led to a bright colour and appearance change. On the other hand, at lower EW concentrations (50, 100 and 200 mg L⁻¹), the lenticels were damaged by the growth of *B. cinerea* due to insufficient effects of the EW treatments. On the other hand, the study demonstrated that EW treatment (300 mg L⁻¹) was sufficient to control appearance throughout the storage time at different treatment time. Chen et al. (2020) reported that acidic EW (80 mg L⁻¹) treated (10 min) and stored (25 °C for 6 days) 'Fuyan' longans had higher commercial acceptability than the control since acidic-EW treatment could reduce the change of appearance colour and retain higher nutritive properties of fruit during postharvest storage. Similarly, Hayta and Aday (2015) reported that EW treatments concentration above 200 mg L⁻¹ had a negative impact on the sensory quality of sweet cherry stored for 30 days under passive atmosphere packaging at 4 °C. These finding indicated that identifying optimum type and concentration of EW for a particular fruit cultivar to maintain the visual appearance with suitable effects towards microbial inactivation is very crucial.

3.2.2. Efficacy of electrolyzed water treatment against *B. cinerea*

The results obtained showed that electrolyzed water treatment (SAI-EW) concentration, dipping duration and change in storage duration had a significant effect on the lesion area of *B. cinerea* (Figure 2). After two weeks of storage, the lesion area doubled from the initial growth area for EW-1 and EW-2, in all the dipping durations (5, 10 and 15 min). For instance, the growth area for the lower SAI-EW concentration (50 mg L⁻¹) was 93%, 67% and 87% for 5, 10 and 15 min, respectively. Similar result was obtained for SAI-EW concentration 100 mg L⁻¹, wherein the recorded growth area was 90%, 66% and 78% for 5, 10 and 15 min,

respectively. Significantly smaller lesion area was observed when the EW concentrations increased above 200 mg L⁻¹ and the curative efficacy was most effective at concentration of 500 mg L⁻¹, followed by 400 mg L⁻¹, and 300 mg L⁻¹ for treated apples.

Furthermore, varying dipping duration (5, 10 and 15 min) at did not have a significant ($P < 0.05$) difference to control the growth of *B. cinerea*. The highest lesion area with *B. cinerea* growth were observed for non-treated control, EW-1 and EW-2 treated fruit stored 5 °C. This study showed that SAI-EW concentration below 200 mg L⁻¹ was not effect as a curative strategy for the managements of *B. cinerea*. However, comparing the results under the lowest EW concentration (50 mg L⁻¹) to the control non-treated apples at the end of storage, the growth of *B. cinerea* under control apple sample were 52%, 88% and 66% higher than apples treated at 5, 10 and 15 min, respectively. Guentzel et al. (2010) demonstrated the effect of near neutral electrolyzed water (50–100 mg L⁻¹) to inactivate *B. cinerea* for peaches and grapes resulted in 10⁶ reductions and 100% inactivation as well as EW did not leave any chlorine residue on the fruit surface comparing water dipped.

Change in storage temperature from cold storage (5 °C) to shelf life (24 °C) was observed to accelerate the growth of *B. cinerea* (Figures 3 and 4). The mean initial lesion areas on the EW treated and control apple at 24 °C were ranges of 69.36–90.36 mm for 5 min, 47.87–107.4 mm for 10 min and 49.91–148.34 mm for 15 min treatment times, whereas the initial lesion area for control fruit were 69.35 mm. From the EW treated fruit, the highest growth area of *B. cinerea* was for 50 mg L⁻¹ for 5 min and 10 min treatment time after two-day storage at 24 °C for 14 and 21-days storage duration. The lesion area increases >80%, >75% and >70% for 5, 10 and 15 min EW-1 and EW-2 treated fruit and stored at 24 °C for 21 days, respectively (Figure 4). However, for similar storage duration, the lesion area for the control fruit increases more than 95%. These results clear showed that the EW treatment could be used to inhibit the growth of *B. cinerea* even at shelf life storage (24 °C). The increase in the lesion area during the storage could be associated with the decrease in the efficacy of EW during with time, which is associated with the change in the property of EW. According to Rahman et al. (2016) the



Figure 3. Differences in lesion area inoculated with *B. cinerea* on 'Granny Smith' apples: non treated (A); treated with different concentrations (EW-1 to EW-6) of electrolyte water treatment for 5 min (B), for 10 min (C) and for 15 min (D) after four weeks storage at 5 °C.

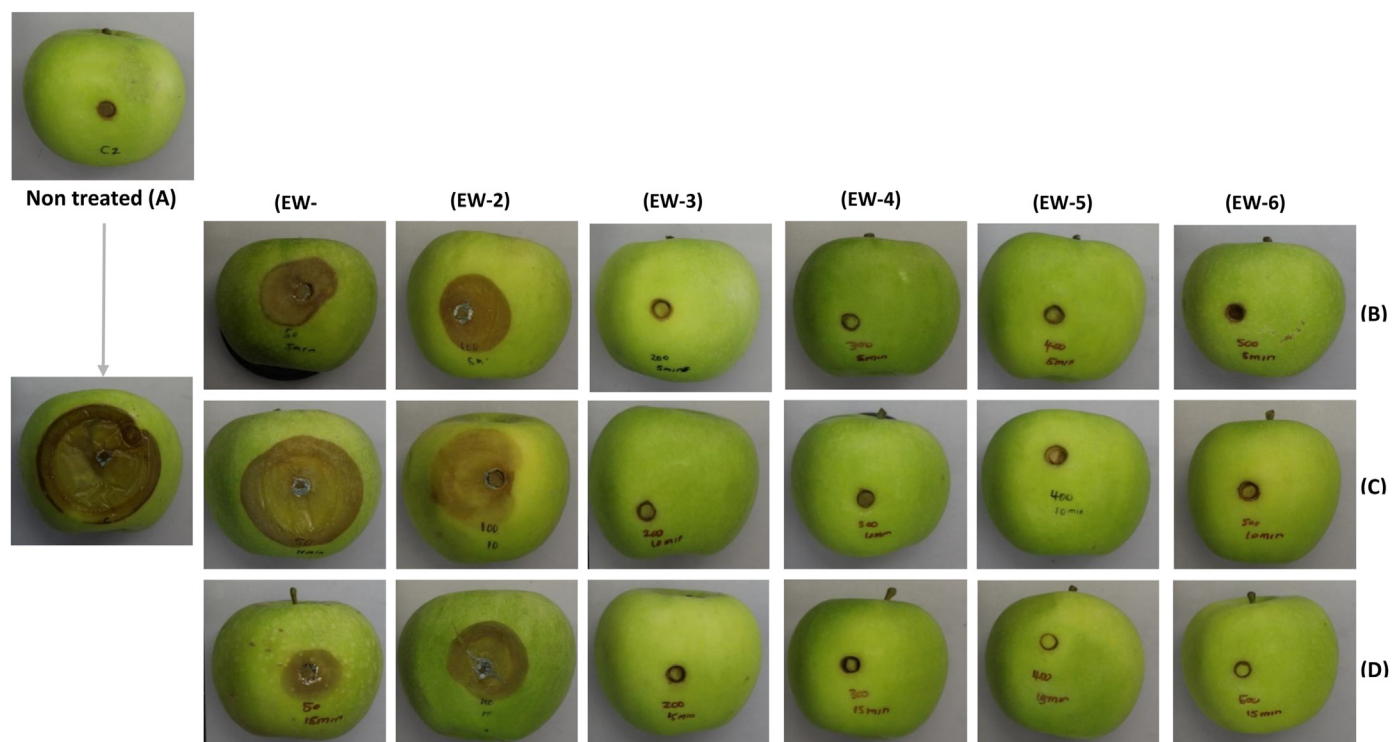


Figure 4. Differences in lesion area inoculated with *B. cinerea* on 'Granny Smith' apples: non treated (A); treated with different concentrations (EW-1 to EW-6) of electrolyte water treatment for 5 min (B), for 10 min (C) and for 15 min (D) after four weeks storage at 24 °C.

antimicrobial efficacy of EW is highly influenced by ORP and PH. Higher ORP of EW could leads to oxidation, affecting various layer of cells, causing the oxidation of sulfhydryl mixture of cell surface and disturbing metabolic pathways inside the cell.

In the study done by [Guentzel et al. \(2010\)](#), a 10 min EW treatment of green table grapes inoculated with *B. cinerea* resulted in a 1% incidence of infection and a decrease severity rating of 2% after 10 days of storage at 25 °C. Similarly, slightly acidic EW (80 mg L⁻¹) treated 'Fuyan'

longans fruit stored at 25 °C showed 22% lower disease index than control fruit by day 6 (Chen et al., 2020). This study further demonstrated the effectiveness of acidic EW treatment with pH of 2.5 towards maintaining the quality attributes and nutritive properties of the fruit. According to Sheng et al. (2020), 100 mg L⁻¹ neutral EW with 2 min contact time showed limited efficacy towards inactivation of *L. monocytogenes* in fresh ‘Granny Smith’ and ‘Fuji’ apple stored for 48 h in ambient condition. The study further stated that, the efficacy of EW could be influenced by fruit surface morphology, EW concentration and treatment contact time, which longer contact time might increase microbial resistance, binding strength of bacteria to the produce surface or might induce bacterial desiccation stress response. This could explain the beneficial effect of 10 min treatment time in comparison of 5 and 15 min treatment time. Studies are evident that EW does not have negative impact on human health since EW can be converted back to ordinary water when there is a contact with organic matter or when diluted with tap water (Hayta and Aday, 2015).

4. Conclusion

The study explored an alternative postharvest pre-treatment strategy using hot water (HW) and slightly alkaline electrolyzed water (SAI-EW) for maintaining quality and managing decay of ‘Granny Smith’ apples. The effects of HWT dipping showed that the treatment at 45 °C dipped for 15 min maintained the physicochemical quality attributes. Electrolyzed water treatments had significant curative effects against the growth of *B. cinerea*. However, based on the outcomes from this study, dipping ‘Granny Smith’ apples in SAI-EW (with varying concentration of 50, 100, 200, 300, 400 and 500 mg L⁻¹) beyond 10 min did not confer any additional benefits. The EW treatments at lower concentrations (50 and 100 mg L⁻¹) at different dipping duration were not able to control the growth of *B. cinerea* during cold storage. However, SAI-EW at 200 mg L⁻¹ combined with cold storage was effective in retarding the growth of *B. cinerea*. These results indicated the importance of selecting appropriate hot water dipping duration. In addition, the findings demonstrate the potential of slightly acidic electrolyzed water as an alternative fruit decay management strategy. Further study is required to elucidate on the impact of this treatment on nutritional, sensorial and functional properties of ‘Granny Smith’ apples.

Declarations

Author contribution statement

Nandi E. Nyamende, F. R. Domtchouang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zinash A. Belay: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zanephy Keyser, Ayodeji Oyenih: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Oluwafemi J. Caleb: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare the following conflict of interests: Oluwafemi J. Caleb; [is an Advisory Board Member for Heliyon Food Science and Nutrition].

Additional information

No additional information is available for this paper.

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