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Original article

Impacts of alkaline-electrolyzed water treatment on physicochemical, phytochemical, antioxidant properties and natural microbial load on 'Granny Smith' apples during storage

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Summary Providing consumers with fresh and safe fruit can greatly increase their access to recommended daily intake of phytonutrients. Thus, this study investigated the impact of dipping in alkaline electrolyzed water (AEW) and dipping duration on the physicochemical, phytonutrients and microbial load on 'Granny Smith' apples. Apples treated with AEW (200 and 300 mg L^{-1}) were compared to sodium hypochlorite (NaOCl, 200 mg L^{-1}) and to non-treated (control) under dipping durations of 10 and 15 min. Apples treated with AEW maintained titratable acidity (TA) and total soluble solids (TSS) compared to other treatments ($P > 0.05$). Interaction of treatments and storage duration had a significant impact ($P \leq 0.05$) on total phenolics and flavonoids contents. Apples treated with AEW better maintained antioxidant capacity compared to control. Treatments with AEW resulted in ≈2 Log reduction in total aerobic mesophilic bacteria (4.1 Log CFU cm^{-2} to 2.2 Log CFU cm^{-2}) and < 1 Log reduction for yeasts and moulds (3.9 Log CFU cm^{-2} to 2.7 Log CFU cm^{-2}). This study demonstrated the efficacy of electrolyzed water treatment as an alternative to conventional pack-house practices for apple fruit.

Keywords Antioxidant capacity, flavanols, food safety, *Malus domestica*, total phenolics.

Introduction

Apples (*Malus domestica*) are rich in dietary fibre, polyphenols, flavonoids and antioxidant activities with great potential to contribute to human health (Lee *et al.*, 2003; Musacchi & Serra, 2018; Shen *et al.*, 2021). However, apples are highly perishable and susceptible to various postharvest diseases, which could lead to loss of quality during storage. Over the years, chlorine at concentration range (50–200 mg L^{-1}) is widely used as disinfectants for washing fresh produce (Aryal & Muriana, 2019). Oxidative reaction of chlorine species present in chlorine-based sanitisers is powerful compared to non-chlorine-based sanitisers. However, the role of chlorine and its toxic end-products have raised public health concerns, resulting in additional regulatory barriers for the use of chlorine and strict residual levels (Ayebah & Hung, 2005; Nan *et al.*, 2019). Therefore, it is necessary to develop alternatives that are environmentally friendly, relatively low cost and effective such as electrolyzed water (EW). Over the last 10 years, the cost of hypochlorites have increased by an average of 10% year on year. In addition, due to the cost of dechlorination the total cost of using chlorinated water treatment could increase by about 50% (U.S. EPA, 1999). Similarly, chlorine dosage varies based on chlorine demand (U.S. EPA, 1999), operating cost is further escalated due to higher dosage. However, the cost of electrolyte generating reactor costs have stayed relatively constant, hence, it is now price competitive. Furthermore, based on our in-house cost analysis, for a medium-sized packhouse with monthly spend of $900–$1500 for chemicals, a straight swap to using an electrolyte rector will have return on investment within 18 months.

Electrolyzed water (EW) is generated via electrolysis of sodium chlorine brine solution, which can change

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its available chlorine concentration (ACC), oxidation–reduction potential (ORP) and pH (Huang *et al*., 2008; Chen *et al*., 2017; Nyamende *et al*., 2021). Alkaline electrolysed water (AEW) is formed at the cathode side of the electrolysis chamber where positive ions such as H^+ and Na^+ move towards the cathode where they take electrons and become hydrogen gas (H_2) or sodium hydroxide (NaOH) (Huang *et al*., 2008). Alkaline electrolysed water (AEW) has a high pH ranging from 11.0-13, low ORP ranging from −795 to −900 mV, lower dissolved oxygen, and high active hydrogen than tap water or deionised water (Shimamura *et al*., 2016). The pH of AEW plays a role in disinfection as it is greater than 11 and the presence of dilute NaOH has been linked with a high surface decontamination effect.

Postharvest application of EW on whole and fresh-cut apples has been extensively focused on managing microbial load or reducing fungal pathogens (Kim *et al*., 2000; Chen *et al*., 2019; Graça *et al*., 2020), impact of sensory quality (Nimitkeatkai & Kim, 2009) and reduction in pesticide residues on 'Cripps Pink' apples (Ferri *et al*., 2016). Higher flavonoid contents was reported for sprout treated with EW compared to NaOCl and tap water treated samples at 4 °C for 7 days (Zhang *et al*., 2019).

Thus, the set hypothesis for this study was that varying the concentration of EW and dipping duration would enhance antioxidant properties, boost accumulation of phenolics and flavonoids, and reduce surface microbial load on 'Granny Smith' apples. The objectives of this study were to evaluate the: (i) impacts of EW on fruit physicochemical attributes and phytonutrients (antioxidant activity, phenolics, total flavanol and flavonol content) of 'Granny Smith' apples; and (ii) effects of EW treatments in reducing aerobic mesophilic bacteria and yeasts and moulds on the fruit surface.

Materials and methods

Plant material and electrolyzed water preparation

Fresh 'Granny Smith' apples (*M. domestica*) were harvested at commercial maturity from the Agricultural Research Council (ARC) Elgin Research Farm, Grabouw, South Africa. Maturity index was based on average total soluble solids (TSS) and titratable acidity of 10.7 ± 0.17°Brix and 0.9 ± 0.04 g $100\ mL^{-1}$, respectively. Harvested fruit were transported in cool trucks (4 °C) from the farm to Postharvest iQ Laboratory, ARC Infriutec-Nietvoorbij, Stellenbosch, where they were sorted upon arrival. Only healthy fruit were selected and stored under regular atmosphere (RA) at 0.5 °C for 3 months (to mimic pack-house long-term storage) prior to the start investigation. Apples were not treated with ethylene inhibitors to micmic pack-house short-term sales practices. Fruit were divided into three batches and each containing 330 fruit per batch, with each batch representing a treatment. Each treatment consisted of ten fruit per corrugated box (400 × 300 × 82 mm) repeated in triplicate, and stored at 5 °C, 95% RH in a sensor monitored cold room.

Alkaline electrolyzed water (AEW) was generated using the ELA-12 000ANW system (ECA Technologies, Envirolyte, South Africa) with sodium chloride salt. The AEW produced consisted: available chlorine concentration (ACC) of 500 mg L^{-1}, ORP > −800 mV, and pH = 11–13. AEW solutions was diluted to the desired concentrations (200–300 mg L^{-1}) prior to dipping the apples. For the standard pack-house practice, food grade NaOCl (11.5% M/V) were procured from Protea Chemicals (Sandton, South Africa) with ACC of 500 mg L^{-1} and pH = 6.5–7.5, and was diluted to 200 mg L^{-1}. Apples were treated by dipping in AEW, NaOCl solution (as positive control), and non-treated samples as negative control (Table 1). Dipping duration selected was based on the average pack-house processing time for apples. After treatments all apples were stored under RA at 5 °C and 90 ± 2% RH for 21 days. Samples were taken for analysis on day 0, 7, 14 and 21.

Physiochemical parameters

Tissue strength and colour

Tissue strength of the fruit were measured using a texture analyser (FTA 20, Güss, South Africa) according to a method described by Nsumpi *et al*. (2020). Each fruit was placed on the texture analyser for a compression test using a probe set with 11.1-mm diameter at a penetration speed of 10 mm s^{-1} and a depth of 8.9 mm. Tissue strength was expressed in Newton (N). Colour changes on each apple fruit were measured

Table 1 Description of treatments and abbreviations used in the study

Treatment(s)			
Active compound	**Concentration (mg L^{-1})**	**Dipping duration (min)**	**Treatment(s) abbreviation**
Non-treated	–	–	Control
NaOCl	200	10	C1
NaOCl	200	15	C2
AEW	300	10	EW1
AEW	300	15	EW2
AEW	200	10	EW3
AEW	200	15	EW4

AEW, alkaline electrolyte water; NaOCl, Sodium hypochlorite.

based on the Commission International del' Eclairage (CIE) colour system using a digital Chroma-meter (CR 400/410 Konica Minolta Sensing Inc., Osaka, Japan). Measurements were taken from opposite sides each fruit (n = 10). Colour calibration of the chroma-meter was performed prior to each measurement. L^* denoting lightness, a^* as red (+)/green (−) and b^* describes yellow (+)/blue (−) were measured. Chorma (C^*) and $h°$ angles were calculated (Belay *et al*., 2017).

Total soluble solids (TSS) and titratable acidity (TA)
From each treatment, apples were processed into juice using a juice extractor (4294 J700; Braun, Shanghai, China). TSS of the juice was measured using a calibrated hand-held refractometer (Pal-1, Atago Co. Ltd., Tokyo, Japan). TA was measured by titrating juice samples against 0.333 N sodium hydroxide to a pH point of 8.2, using Crison Titromatic 1S/2B (Crison Instruments, Barcelona, Spain). TSS was expressed as °Brix, while TA was expressed as g 100 mL^{-1} of malic acid (Nsumpi *et al*., 2020). Thereafter, juice samples were stored at − 80 °C prior to further analysis. On the day of analysis, samples were removed from the freezer and allowed to thaw at room temperature (20 °C) before they were centrifuged at 2951 ***g*** using Hermle Z206A compact centrifuge (Wehingen, Germany). Supernatants were then used for phytonutrient and antioxidant evaluations.

Total phenolics, total flavonols and flavanols, and antioxidant capacity

Total phenolic concentration (TPC) was measured using Folin-Ciocalteu method as described by Belay *et al*. (2017). Reaction plate was allowed to stand for 2 h at room temperature (20 °C) in the dark before reading. Absorbance was read at 750 nm using microplate spectrophotometer (Fluostar Omega, BMG Labtech, Offenburg, Germany). Gallic acid equivalent (GAE) standard curve was used to extrapolate TPC and expressed as mg GAE L^{-1}. Total flavonol content (TFC) was determined according to a slightly modified method described by Pavun *et al*. (2018). TFC was expressed as mg quercetin equivalent (QE) L^{-1}. Total flavanol content (TFAC) was estimated using a modified *p*-dimethylaminocinnamaldehyde method (Wang *et al*., 2015). TFAC was calculated from a calibration curve using catechin as the standard. Total flavanol was expressed as mg catechin equivalents (CE) L^{-1}. Trolox equivalent antioxidant capacity (TEAC) assay based on 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical cation ($ABTS^+$) previously described by Duda-Chodak *et al*. (2011) was used. Absorbance reading of the reaction mixture was taken at 734 nm using the microplate spectrophotometer. Antioxidant capacity was expressed as mg TE L^{-1} of apple juice.

Microbial analysis

Samples were analysed for total aerobic mesophilic bacteria (TAMB), yeasts and moulds by total plate count method (Caleb *et al*., 2013). The surface area (≈90 cm^2) of 'Granny Smith' apples was estimated based on the approach described by Galbreath (1976). Each whole apple was put into an aseptic physiological saline (PS) solution and slowly vortex for 60 min to remove the microbial load on the fruit surface. Three-fold dilutions were prepared using 1.0 mL of diluents into 9.0 mL of PS. To enumerate microbial load, 1.0 mL of each dilution was pour plated onto appropriate media. Plates were incubated 3–5 days at 28 °C for yeasts and moulds, and 2 days at 37 °C for TAMB. The number of colony forming unit per square centimetre of surface (CFU cm^{-2}) was calculated and transformed to Log CFU cm^{-2}.

Statistical analysis

Factorial analysis of variance (ANOVA) was used to elucidate the impacts of treatments, dipping time and storage duration on measured attributes of 'Granny Smith' apples at 95% confident interval using Statistica Software (version 13, StatSoft Inc., Kraków, Poland, TIBCO Software Inc., Palo Alto, CA, USA). Duncan multiple range test was used to determine the difference between means. Correlation analysis was perfomed using XLSTAT software version 2016.06.37085 (Microsoft, New York, NY, USA). Results were presented as mean ± standard deviation.

Results and discussion

Physiochemical parameters

Tissue strength
The interactions of treatments, dipping and storage duration had no significant impact ($P > 0.05$) on the tissue strength of apples (Table 2). On day 21, samples treated with chlorinated water (C2, 200 mg L^{-1} for 15 min) had highest tissues strength (6.46 ± 0.85 N), while control had the lowest (5.13 ± 0.22 N) tissues strength. This observation on texture profile is in agreement with the report by Ding *et al*. (2015) and Hung *et al*. (2010). Changes in cell wall biopolymers, in particular the pectin may impair cell wall structure and result in tissue softening (Harker *et al*., 2006; Chen *et al*., 2017). Furthermore, epidermal layers of a mature apple fruit consist of three distinct layers: (i) the waxy layer (with partially crystalline character), (ii) the cutin layer, and (iii) cell walls. Wax layer is believed to be the main barrier limiting mass transport to-and fro the fruit tissue (Veraverbeke *et al*., 2001). Hence, it is believed that the wax

Table 2 Effect of different treatments on physical and biochemical quality attributes of cv. 'Granny Smith' apple stored at 5 °C and 90 ± 2% RH for 21 days

Treatment (s)[†]	Storage duration (days)	Tissue strength (*N*)	Colour parameters					TSS (°Brix)	TA (g 100 mL^{-1})
			*L**	*a**	*b**	*C**	*h*°		
Control	0	5.5 ± 0.35^{ab}	50.1 ± 6.87^{abc}	−20.1 ± 5.44^{ab}	37.9 ± 2.31^{ab}	40.9 ± 7.52^{ab}	104.8 ± 10.69^{c}	12.7 ± 0.31^{bc}	1.2 ± 0.03^{ab}
	7	5.7 ± 0.24^{a}	54.8 ± 2.31^{ab}	−13.0 ± 2.44^{bc}	35.7 ± 2.11^{b}	37.4 ± 4.80^{b}	105.6 ± 7.6^{c}	13.4 ± 0.50^{ab}	1.0 ± 0.04^{c}
	14	5.7 ± 0.30^{ab}	55.6 ± 2.30^{ab}	−13.3 ± 2.69^{bc}	38.8 ± 2.31^{ab}	40.6 ± 5.60^{ab}	108.5 ± 5.40^{bc}	12.9 ± 0.23^{bc}	1.0 ± 0.07^{c}
	21	5.1 ± 0.21^{b}	48.0 ± 2.49^{c}	−4.4 ± 1.17^{d}	28.5 ± 3.92^{c}	37.9 ± 3.70^{b}	110.9 ± 3.80^{ab}	12.5 ± 0.26^{c}	0.9 ± 0.15^{c}
C1	0	5.5 ± 0.35^{ab}	50.1 ± 6.87^{abc}	−20.1 ± 5.44^{ab}	37.9 ± 2.31^{ab}	40.9 ± 7.52^{ab}	104.8 ± 10.69^{c}	12.7 ± 0.31^{bc}	1.2 ± 0.03^{a}
	7	5.7 ± 0.22^{a}	53.1 ± 2.28^{ab}	−14.5 ± 2.43^{bc}	38.1 ± 1.99^{ab}	41.8 ± 2.67^{a}	110.18 ± 3.05^{ab}	12.6 ± 0.42^{bc}	1.1 ± 0.03^{bc}
	14	5.8 ± 0.41^{ab}	51.8 ± 1.61^{bc}	−10.2 ± 2.09^{cd}	36.1 ± 1.92^{ab}	40.9 ± 7.52^{ab}	104.8 ± 10.69^{c}	12.8 ± 0.20^{bc}	0.9 ± 0.11^{c}
	21	5.5 ± 0.21^{ab}	53.9 ± 2.89^{ab}	−14.1 ± 2.32^{bc}	39.6 ± 2.43^{ab}	43.9 ± 4.98^{a}	111.4 ± 2.27^{ab}	12.4 ± 0.49^{bc}	0.7 ± 0.12^{c}
C2	0	5.5 ± 0.35^{ab}	50.1 ± 6.87^{abc}	−20.1 ± 5.44^{ab}	37.9 ± 2.31^{ab}	40.9 ± 7.52^{ab}	104.8 ± 10.69^{c}	12.7 ± 0.31^{bc}	1.2 ± 0.03^{a}
	7	5.9 ± 0.25^{a}	53.7 ± 2.23^{ab}	−14.4 ± 1.80^{ab}	38.5 ± 1.71^{ab}	43.5 ± 3.05^{a}	112.3 ± 3.79^{ab}	12.6 ± 0.40^{bc}	1.1 ± 0.04^{bc}
	14	5.8 ± 0.32^{a}	54.5 ± 1.98^{ab}	−12.1 ± 2.51^{bc}	36.9 ± 2.18^{ab}	41.7 ± 2.29^{a}	109.2 ± 4.40^{ab}	12.4 ± 0.15^{c}	0.8 ± 0.12^{cd}
	21	6.5 ± 0.85^{a}	54.1 ± 2.69^{ab}	−14.0 ± 2.32^{bcd}	38.5 ± 2.75^{ab}	41.6 ± 4.67^{ab}	109.4 ± 4.24a^{bc}	12.5 ± 0.10^{c}	0.7 ± 0.05^{d}
EW1	0	5.5 ± 0.35^{ab}	50.1 ± 6.87^{abc}	−20.1 ± 5.44^{ab}	37.9 ± 2.31^{ab}	40.9 ± 7.52^{ab}	104.8 ± 10.69^{b}	12.7 ± 0.31^{bc}	1.2 ± 0.03^{a}
	7	5.4 ± 0.25^{ab}	53.8 ± 2.25^{ab}	−14.4 ± 2.22^{bcd}	38.6 ± 1.84^{ab}	42.1 ± 3.84^{a}	109.7 ± 4.26b^{c}	12.7 ± 0.44^{bc}	1.1 ± 0.04^{bc}
	14	5.7 ± 0.32^{ab}	52.1 ± 4.54^{abc}	−10.3 ± 1.92^{d}	38.1 ± 2.26^{ab}	36.3 ± 6.44^{b}	103.3 ± 10.74^{c}	12.6 ± 0.20^{bc}	0.9 ± 0.14^{c}
	21	5.6 ± 0.29^{ab}	56.2 ± 2.02^{ab}	−13.7 ± 2.41^{bc}	40.2 ± 2.02^{a}	43.1 ± 2.39^{a}	109.5 ± 3.63^{b}	12.3 ± 0.51^{c}	0.7 ± 0.15^{cd}
EW2	0	5.5 ± 0.35^{ab}	50.1 ± 6.87^{abc}	−20.1 ± 5.44^{abc}	37.9 ± 2.31^{ab}	40.9 ± 7.52^{ab}	104.8 ± 10.69^{c}	12.7 ± 0.31^{bc}	1.2 ± 0.03^{ab}
	7	5.6 ± 0.24^{ab}	57.2 ± 2.19^{a}	−15.5 ± 2.20^{ab}	40.1 ± 2.21^{a}	41.0 ± 4.16^{ab}	108.3 ± 6.21^{bc}	12.7 ± 1.06^{abc}	1.0 ± 0.08^{bc}
	14	5.7 ± 0.31^{ab}	54.9 ± 2.92^{ab}	−14.2 ± 2.53^{bcd}	38.8 ± 2.28^{ab}	42.6 ± 3.70^{a}	109.4 ± 3.03^{b}	12.6 ± 0.21^{bc}	1.0 ± 0.09^{bc}
	21	6.0 ± 0.31^{a}	54.9 ± 2.22^{ab}	−15.1 ± 3.10^{bcd}	39.5 ± 2.57^{ab}	42.2 ± 5.70^{ab}	107.1 ± 70.90^{b}	12.7 ± 0.21^{bc}	0.7 ± 0.12^{cd}
EW3	0	5.5 ± 0.35^{ab}	50.1 ± 6.87^{abc}	−20.1 ± 5.44^{abc}	37.9 ± 2.31^{ab}	40.9 ± 7.52^{ab}	104.8 ± 10.70^{c}	12.7 ± 0.31^{bc}	1.2 ± 0.03^{ab}
	7	5.9 ± 0.22^{a}	54.1 ± 1.60^{ab}	−14.1 ± 1.98^{bc}	37.7 ± 1.36^{ab}	40.4 ± 3.51^{ab}	109.3 ± 3.27^{b}	13.0 ± 0.15^{ab}	1.1 ± 0.07^{bc}
	14	6.0 ± 0.35^{a}	53.1 ± 3.11^{abc}	−12.0 ± 2.42^{cd}	38.5 ± 2.07^{ab}	41.3 ± 9.14^{ab}	103.8 ± 12.13^{c}	12.2 ± 0.40^{c}	1.0 ± 0.11^{bc}
	21	5.6 ± 0.25^{ab}	55.3 ± 2.63^{ab}	−14.9 ± 1.64^{b}	40.3 ± 2.18^{a}	42.9 ± 4.53^{ab}	110.4 ± 5.14^{ab}	12.2 ± 0.35^{c}	0.8 ± 0.08^{c}
EW4	0	5.5 ± 0.35^{ab}	50.1 ± 6.87^{abc}	−20.1 ± 5.44^{ab}	37.9 ± 2.31^{ab}	40.9 ± 7.52^{ab}	104.8 ± 10.7^{c}	12.7 ± 0.31^{bc}	1.2 ± 0.03^{a}
	7	5.7 ± 0.39^{ab}	55.0 ± 1.77^{ab}	−14.3 ± 1.26^{b}	38.2 ± 1.46^{ab}	41.1 ± 3.15^{ab}	110.5 ± 4.64^{ab}	13.3 ± 0.40^{ab}	1.1 ± 0.06^{b}
	14	6.1 ± 0.30^{a}	51.8 ± 4.45^{abc}	−13.5 ± 2.03^{bc}	38.1 ± 1.89^{ab}	37.1 ± 12.07^{b}	110.9 ± 6.06^{ab}	13.3 ± 0.90^{abc}	1.0 ± 0.07^{bc}
	21	5.8 ± 0.29^{a}	54.9 ± 3.27^{ab}	−14.2 ± 2.04^{bc}	39.6 ± 2.43^{ab}	39.8 ± 6.68^{b}	104.9 ± 6.98^{c}	12.4 ± 0.52^{bc}	0.8 ± 0.04^{d}

Mean values ± standard deviation (SD) tested using Duncan multi-range test at 95% confident interval ($P < 0.05$). Similar lower case letters are not significantly different.
[†]The descriptions of treatments: C1 = NaOCl (200 mg L^{-1}/10 min); C2 = NaOCl (200 mg L^{-1}/15 min); EW1 = AEW (300 mg L^{-1}/10 min); EW2 = AEW (300 mg L^{-1}/15 min); EW3 = AEW (200 mg L^{-1}/10 min); EW4 = AEW (200 mg L^{-1}/15 min).

layer of the apple cuticle prevented wetting of fruit tissue.

Colour

A slight increase in L^* values across all treatments was observed as the storage period progressed compared to day 0, but, these increases were not statistically different ($P > 0.05$) (Table 2). Highest L^* (56.2 ± 2.13) value was observed for EW2 samples, while control samples had the least L^* (48.0 ± 2.42) on day 21. A similar observation was reported by Chen *et al.* (2020) for longan fruit. Furthermore, a^* and b^* values for all the treatments groups were not significantly affected by the interaction of treatments and storage days ($P > 0.05$). In addition, AEW treatments maintained initial C^* value till the end of storage, except in control and EW4 samples where the values decreased slightly. A progressive increase in $h°$ was observed with the increase in storage duration for all samples (Table 2).

Colour of fruit reflects maturity and freshness, hence, maintenance of colour is vital during storage to ensure marketability and consumer acceptance. Other studies found no significant impact of EW treatment on colour attributes for other fresh produce (Youssef & Hussien, 2020). Apple peel colour is influenced by various pigmentations such as phenolics (Musacchi & Serra, 2018). The TP of apples was well maintained in this study, which could account for the minimal colour changes.

Total soluble solids (TSS) and titratable acidity (TA)

TSS fluctuated throughout the study and statistical analyses showed that interactions of treatment and storage duration had no significant effect ($P > 0.05$). TSS value of fruit increased for control (13.6°Brix) on day 7, and thereafter declined (Table 2). Initial increase TSS during storage could be attributed to hydrolysis of polysaccharides due to the activity of the cell wall degrading enzymes (Bal & Celik, 2010). Fluctuation and decline in TSS could be attributed to intrinsic variation in the fruit metabolic process at the different intervals (Chen *et al.*, 2020). AEW treatment maintained TSS in 'Granny Smith' apples.

On the other hand, the interaction of treatment and storage duration had a significant effect on the TA content of apples ($P \leq 0.05$). A continuous and significant decline in TA was observed across all samples until the end of storage (Table 2). At the end of storage lowest TA content ($0.68\ g\ 100\ mL^{-1}$) was observed in apple treated with chlorinated water for 15 min. Compare to the control samples treatments EW1 to EW4 did not have significant impact. These results are consistent with previous studies, showing that using EW did not have considerable impact on TA (Hung *et al.*, 2010). Organic acids content in fruit directly influence TA. Organic acids are easier to consume as energy source in fruit respiration cycle compared to fatty acids and carbohydrates during postharvest (Serrano *et al.*, 2003). TA decline in this study could be attributed to metabolic processes of apples during storage and consumption of organic acids.

Total phenolics, flavonols and flavanols

Storage duration was found to have a significant effect on TPC of all treated apples ($P \leq 0.05$), as concentration increased continuously across all treatments (Fig. 1a). TPC of apples increased from 709 ± 174.7 mg GAE L^{-1} fresh weight on day 0 to range from 1030 to 1412 mg GAE L^{-1} on day 21. This result agrees with other studies (Li *et al.*, 2020b). Relative influence of AEW > NaOCl = control on TPC. Increase in phenolic compounds in apples could be associated with the biological activities of phenylalanine ammonium lyase, which is stimulated by ethylene action (Napolitano *et al.*, 2004). In addition, studies have shown that enzymes responsible for degradation of phenolics such as polyphenol oxidase are retards at low storage temperatures (Tomas-Barberan & Espin, 2001). Leja *et al.* (2003) showed a noticeable increase in ethylene in apple fruit stored under RA, and reported an increase in phenolic contents during storage. Apples used in this study were not treated with any form of ethylene inhibitors and stored under RA.

Furthermore, interaction of storage duration and treatments had a significant impact ($P \leq 0.05$) on total flavonol and flavanol content (Fig. 1b, c). Total flavonol content increased from 887 mg QE L^{-1} for all treatment types to ranges of 1272–1507 mg QE L^{-1} at the end of storage. Highest flavonol content was found in the control (1507 ± 124 mg QE L^{-1}) and EW4 treated (1425 ± 57 mg QE L^{-1}) apples on day 21. Total flavanol content of apples increased significantly throughout storage, that is, from 119 to 251 mg CE L^{-1} at the end of storage. These results are consistent with other reports using AEW treatment on fresh produce (Chen *et al.*, 2020; Li *et al.*, 2020b). Maintaining relatively high total flavonoid in fruit during storage might contribute to reducing decay development and enhanced resistance (Zhi *et al.*, 2017).

Flavonoids are known to have pleotropic effects and are directly involved in plant defence against pathogens (Sivankalyani *et al.*, 2016). Since both flavonoids and anthocyanin are synthesised through the same defence pathway of phenylpropanoid; it would be practical to postulate that temperature affects the synthesis of both compounds the same way. Reay (1999) demonstrated that transferring 'Granny Smith' apples from low temperature treatment to a higher

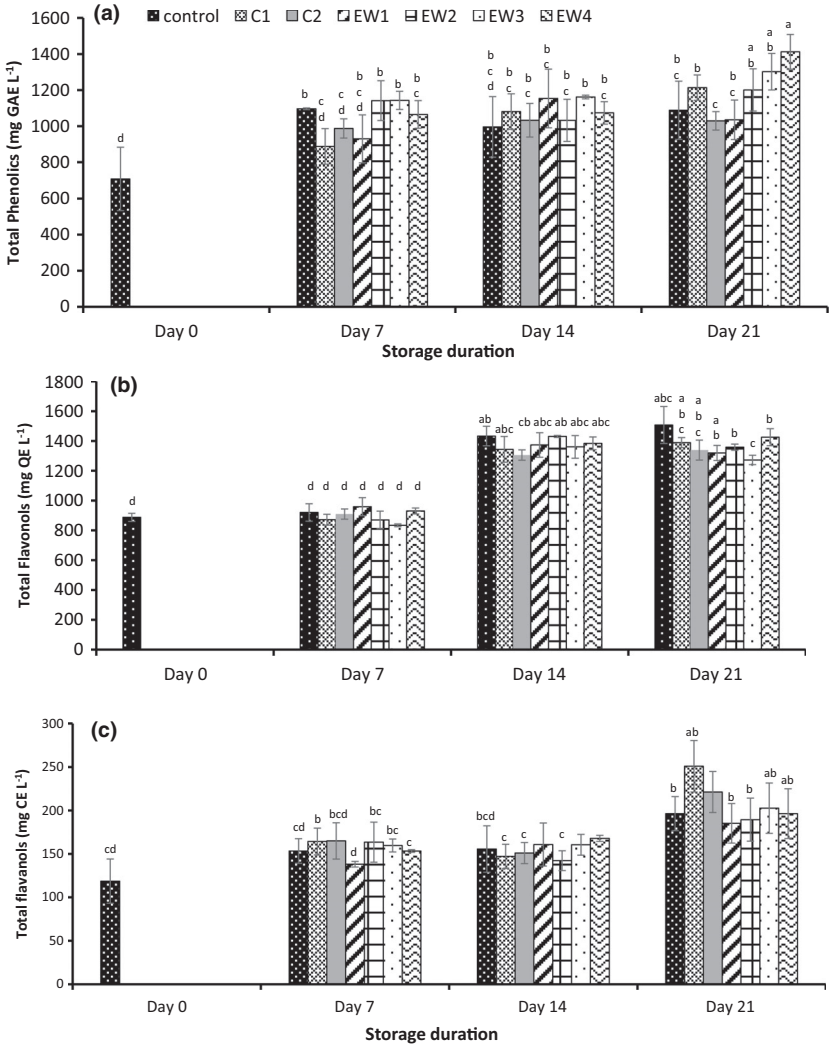

Figure 1 Effects of AEW and chlorinated water treatments on 'Granny Smith' apples: polyphenol concentration (a), total flavonol concentration (b) and total flavanol concentration (c), during storage at 5 °C and 90 ± 2% RH for 21 days. Error bars represent standard deviation (SD) of mean values of treatments (n = 3) tested using Duncan multiple range test at 95% confident interval ($P \leq 0.05$). Similar lower case letters are not significantly different. The descriptions of treatments: C1 = NaOCl (200 mg L^{-1}/10 min); C2 = NaOCl (200 mg L^{-1}/15 min); EW1 = AEW (300 mg L^{-1}/10 min); EW2 = AEW (300 mg L^{-1}/15 min); EW3 = AEW (200 mg L^{-1}/10 min); EW4 = AEW (200 mg L^{-1}/15 min).

temperature significantly enhanced the formation of anthocyanin. Thus, the transfer of 'Granny Smith' apples in this study from 0.5 to 5 °C could have induced the synthesise of flavonoids. Flavonoids also correlate to antioxidant activity in this study (Tables S1–S3). Results demonstrate that AEW treatment combined with storage temperature is capable of delaying degradation of flavonoids and phenolics, and boosted their production in apples during storage.

Antioxidant capacity

TEAC of treated and control apples were significantly influenced by the interactions of treatment types and storage duration ($P \leq 0.05$). A continuous increase in TEAC was observed across all the treatments, besides the control that significantly declined after day 14. On day 21, lowest TEAC was observed for control samples (Fig. 2a), compared to other treated apples. Similarly, other studies have demonstrated that EW treatments maintained antioxidant capacity compared to control samples (Li *et al*., 2020a, 2020b). Overall, AEW treatment maintained a relatively high TEAC levels in 'Granny Smith' apples.

Increase in antioxidant capacity of fruit could be attributed to the accumulation of total phenolics (Leja *et al*., 2003). A significant positive correlation between antioxidant actives and TP content (r = 0.71) as shown in Table S1. In agreement with the results a good correlation between antioxidant capacity and total phenol content was found in several studies (Leja *et al*., 2003; Napolitano *et al*., 2004). However, this relationship between phenolics and antioxidant activity for apples seems to be debatable. Matthes & Schmitz-Eiberger (2009) observed that antioxidant capacity remained unchanged for RA stored 'Pinova' and CA-stored

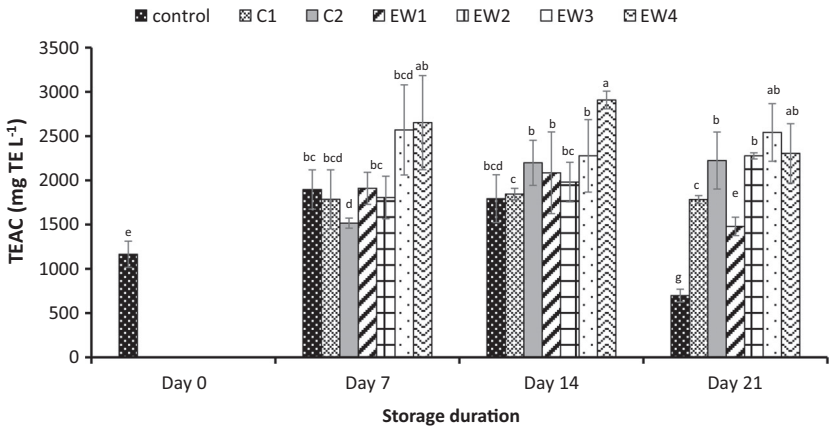

Figure 2 Effects of AEW treatments and chlorinated water treatments on cv. "Granny Smith" apples: Trolox equivalent antioxidant capacity (TEAC) during storage at 5 °C and 90 ± 2% RH for 21 days. Error bars represent standard deviation (SD) of mean values of treatments ($n = 3$) tested using Duncan multi-range test at 95% confident interval ($P \leq 0.05$). Similar lower case letters are not significantly different. The descriptions of treatments: C1 = NaOCl (200 mg L^{-1}/10 min); C2 = NaOCl (200 mg L^{-1}/15 min); EW1 = AEW (300 mg L^{-1}/10 min); EW2 = AEW (300 mg L^{-1}/15 min); EW3 = AEW (200 mg L^{-1}/10 min); EW4 = AEW (200 mg L^{-1}/15 min).

'Golden Delicious' apples, while an increase was reported for other culitvars stored for 4.5 months irrespective of the storage condition. These results evidently confirmed that antioxidant content depends on the cultivar, harvest time, and storage duration.

Microbial load

Results obtained showed that all treatments exhibited significant decontaminating activity against microbial population on the fruit surface (Fig. 3). Compared to control a ≈2 Log reduction in TAMB and <1 Log reduction for yeasts and moulds count was recorded on day 7. Comparison of decontamination efficacy showed that EW4 > EW2 = C1 > control at the end of storage for TAMB. Similarly, for yeasts and moulds count, AEW were more effective compared to conventional chlorine treatments. These findings agree with other studies (Koide *et al*., 2009; Ding *et al*., 2015; Ogunniyi *et al*., 2021). Overall, dipping duration in AEW at a given concentration had no significant impact ($P > 0.05$) on treatment efficacy. According to the South African legislation (FCD Act 54 1979), the maximum limit for yeast and mould and TAMB allowed on fresh fruit is 5 log CFU g^{-1} and 7 log CFU g^{-1}, respectively. At the end storage the highest microbial load in the treated samples were significantly below the maximum limits permitted on fresh fruit, hence, the apples could be considered safe. This suggests that 10 min dipping would be sufficient for the treatment of 'Granny Smith' apples within the packhouse.

Most importantly, AEW treatment showed a strong decontamination efficacy compared to the conventional industry practice. The antimicrobial activity of EW treatment could be attributed to the presence of inorganic oxidants, free reactive chlorine in the form of hypochlorous acid (HClO), ClO^-, and Cl_2, as well as high ORP (Park *et al*., 2004; Liu & Yu, 2017). Previous study showed a high level of correlation between HOCl concentration and the bactericidal efficacy ($r = 0.95$) of EW (Len *et al*., 2000). In contrast, for NaClO solution the key causative factor for antimicrobial action is the hypochlorite ion, which decreases efficacy with increase in organic load. Hypochlorous acid has been shown to be more effective as a sanitising agent than an equivalent concentration of hypochlorite ions (Rahman *et al*., 2010).

Conclusions

Washing either prior to long-term storage or retail distribution is a critical stage in postharvest processing of apples. This study was able to demonstrate that AEW treatment with available chlorine concentrations of 300 and 200 mg L^{-1} were effective at retaining high fruit quality attributes of 'Granny Smith' apples during 21 days of storage at 5 °C. Compared to the control and chlorinated water treatment, the AEW was effective in enhancing and maintaining phytonutrients and antioxidant capacity, with high reduction in microbial load on 'Granny Smith' apples. Electrolyte water dipping offers the potential as an alternative to chlorinate water wash in apple pack-houses. However, further studies on mode of action of EW on fruit surface, and impact of long-term (6–10 months) cold storage of apples on EW efficacy is required.

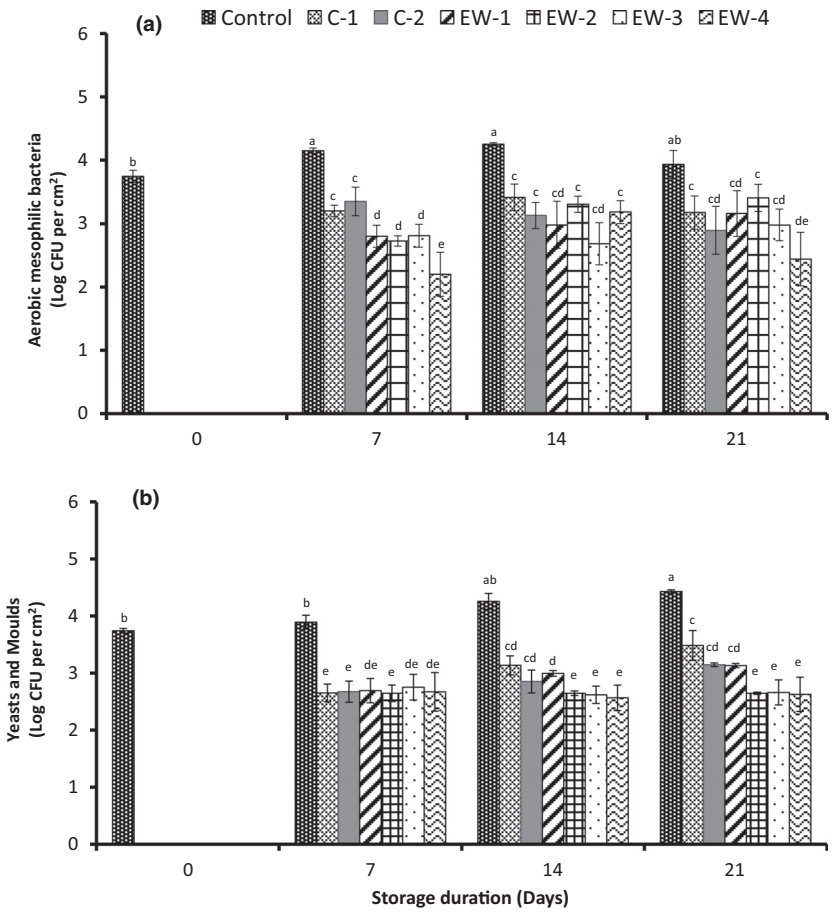

Figure 3 Effects of SAI-EW treatments and chlorinated water treatments on the 'Granny Smith' apple surface microbial load: (a) total aerobic mesophilic bacteria and (b) yeast and moulds, during storage at 5 °C and 90 ± 2% RH for 21 days. Error bars represent standard deviation (SD) of mean values (n = 9) tested using Duncan multi-range test at 95% confident interval ($P \leq 0.05$). Similar lower case letters are not significantly different. The descriptions of treatments: C1 = NaOCl (200 mg L^{-1}/10 min); C2 = NaOCl (200 mg L^{-1}/15 min); EW1 = AEW (300 mg L^{-1}/10 min); EW2 = AEW (300 mg L^{-1}/15 min); EW3 = AEW (200 mg L^{-1}/10 min); EW4 = AEW (200 mg L^{-1}/15 min).

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Conflicts of interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Author contribution

Nandi E. Nyamende: Data curation (lead); Formal analysis (equal); Investigation (lead); Writing-original draft (lead). **Zinash A. Belay:** Data curation (equal); Formal analysis (lead); Funding acquisition (supporting); Investigation (equal); Supervision (equal); Writing-review & editing (equal). **Zanephyn Keyser:** Data curation (supporting); Formal analysis (supporting); Methodology (equal); Software (lead); Supervision (equal); Visualization (lead); Writing-review & editing (equal). **Ayodeji B. Oyenihi:** Methodology (equal); Resources (lead); Software (supporting); Supervision (equal); Validation (lead); Writing-review & editing (supporting). **Oluwafemi James Caleb:** Conceptualization (lead); Methodology (equal); Project administration (lead); Resources (lead); Supervision (lead); Validation (supporting); Writing-review & editing (lead).

Ethical approval

Ethics approval was not required for this research.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary Tables and Figures.