Impact of electrolyzed water treatments on the physiological responses, phytonutrients, and overall quality of 'Granny Smith' apples

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DECLARATION

I, **Nandi Elana Nyamende** the undersigned, hereby declare that the work contained in this thesis is my own original work and that it has not previously, in its entirety or part, been submitted at any other university for a degree. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

'Granny Smith' apples are the main export of pome fruit in South Africa. The fruit are stored for an extended period under normal or controlled atmosphere conditions to take advantage of main export markets such as the United Kingdom (UK) and the European Union (EU). However, during this time the apples are susceptible to various post-harvest pathological disorders such as grey and blue mould, which are mainly due to *Botrytis cinerea* and *Penicillium expansum*, respectively. In addition, 'Granny Smith' apples are susceptible to various storage physiological disorders such as superficial scald.

Current commercial practice to control pathological disorders has been the use of chemical fungicides. However, several fungicides are not used for post-harvest treatment or have been removed from the market due to possible toxicological risks to humans and the environment. Most pack houses in South Africa use a chlorine-based solution prepared commercially from sodium hypochlorite (NaOCl) with concentrations ranging from 5 mg L^{-1} - 200 mg L^{-1} as a decontamination agent for washing the surfaces of fresh produce including apples and has been authorized for use by the fresh produce industry. However, the reactivity of the chlorine species present in chlorine-based sanitizers could be different than compared to other chlorine-based sanitizers because of the reactive and complex nature of chlorine. In addition, the produce industry has raised concerns regarding the additional regulatory barriers, limitations and safety, and regulations for the use of chlorine in its present form. This has therefore made it necessary to urgently investigate alternative non-chemical, safe, environmentally- friendly, and low-cost post-harvest treatment strategies to control the incidence of pathological and physiological disorders in apple fruit. Electrolyzed water (EW) is an emerging hurdle technique and has excellent antimicrobial properties against several microbial pathogens and it is gaining popularity in the food industry. In addition, it has no adverse impacts to the environment. Therefore, the overall aim of this study was to investigate the impact of electrolyzed water treatments on the physicochemical and biochemical quality attributes, as well as changes in phytonutrients and natural microbial load of 'Granny Smith' apples.

Electrolysed water (EW) was effective as a curative agent against *B. cinerea* and *P. expansum*. The slightly alkaline electrolysed water (SAI-EW) treatments against *B. cinerea* resulted in significant ($p \leq 0.05$) reduction in lesion zones of decay across all concentrations in comparison to the control stored up 21 days at 5 °C plus two days of accelerated storage at 24 °C. The curative efficacy of the treatments was most effective at the highest concentration of 500 mg L^{-1}, followed by 400 mg L^{-1}, and 300 mg L^{-1} for treated apples. The

acidic electrolysed water treatments (AEW) against *P. expansum* and *B. cinerea* resulted in a significant ($p < 0.05$) reduction in lesion zones compared to the control samples stored up to 9 days at 15 °C. The AEW curative efficacy was most effective at 300 and 200 mg L^{-1}.

'Granny Smith' apples were further treated with alkaline electrolysed water (AIEW) and compared to sodium hypochlorite at the current industry standard of 200 mg L^{-1}. The impact of these treatments on the overall fruit quality, physiological disorder (superficial scald) and microbial load was investigated. Apples treated with AIEW maintained low pH, titratable acidity (TA) and total soluble solids (TSS) compared to other treatments ($p < 0.05$). The interaction of treatments and storage duration had a significant impact on total polyphenols and total flavonoids ($p \leq 0.05$). At the end of storage day 21, AIEW treated apples better maintained the antioxidant capacity compared to control and sodium hypochlorite (NaOCl, 200 mg L^{-1}) ($p \leq 0.05$). Treatments with AIEW and sodium hypochlorite had no effect on scald incidence. Treatments with AIEW resulted in ≈ 2 Log reduction in total aerobic mesophilic bacteria (from 4.1 Log CFU cm^2 to 2.2 Log CFU cm^{-2}) and < 1 Log reduction for yeast and mould (from 3.9 Log CFU cm^2 to 2.7 Log CFU cm^2) count. At the end of storage, AIEW treated apples (with 200 mg L^{-1} for 15 min) maintained the lowest total aerobic mesophilic bacteria and yeast and mould count compared to the control samples.

Based on the microbial analysis it was expedient to further characterize the microbial community isolated from the AIEW treated and non-treated 'Granny Smith' apples during 21 days post-harvest storage. Based on initial morphological identification about (87) pure colonies (56 possible bacteria and 31 possible yeasts) were isolated across all treated samples from 0 to 21 post-harvest storage. Out of the total 87 isolates, 56 isolates were identified as bacteria via the enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR), and only 27 out of these isolates resulted in genetically diverse bacteria populations. Based on post-harvest treatment selection, a total of eight different dominant bacteria (*Staphylococcus epidermidis, S. capitis, Erwinia aphidicola, Enterobacter bugandensis, Curtobacterium flaccumfaciens, Pseudomonas graminis, Ochrobactrum soli* and *Pantoea agglomerans*) were identified on the surface of treated and non-treated apples via the 16S rDNA. In addition, based on random amplification of polymorphic DNA (RAPD-PCR) using primers 1283, a total of ten fungal isolates were tentatively identified. However, only five out of the ten fungal isolates could be amplified via their ITS1 and ITS4 primers within the 100-1500 bp region. Identified fungi included *Rhodotorula nothofagi*, *Aspergillus inuii*, *Debaryomyces hansenii* and *Phialemoniosis curvata*, which predominated in different treatments.

Results obtained in this study suggest that SAI-EW and AEW can be an effective alternative for the post-harvest management of *B. cinerea* and *P. expansum* during the storage of 'Granny Smith' apples. Due to greater susceptibility to superficial scald, prevention measures such as treatment with 1-Methylcyclopropene (1-MCP) could be applied after EW treatment for long term storage. The overall desirable effects described in this study provides a guiding tool for the fruit industry on the postharvest sanitation alternative for deciduous fruits. The outcome provides an alternative to chlorine-based sanitisers.

Keywords: *Malus domestica*, 'Granny Smith', electrolysed water (EW), antifungal activity, fruit quality, antioxidant capacity, microbial community

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DEDICATION

to my beautiful mother **Xoliswa Nyamende**, and my father **Tycoon Nyamende** for always pushing and believing in me, my daughter **Kai-lee Minenhle Nyamende**, my friends and all those close to my heart, had it not been for their constant love and support I am not sure any of this would be possible

“Delight yourself in the Lord and He shall give you the desires of your heart”

Psalms 37:4

RESEARCH OUTPUTS

The following research outputs represent a list of published and submitted articles, and conference presentations contributed by the candidate to scientific knowledge and development during her MSc candidacy (2019-2021):

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Nandi E. Nyamende[a, b], Rosette R. F. Domtchouang[a], Zinash A. Belay[a], Zanephyn Keyser[b], Ayodeji B. Oyenihi[c], Oluwafemi J. Caleb[a]*. Alternative postharvest pre-treatment strategies for quality and microbial safety of 'Granny Smith' apple, *Heliyon*, **7**(5), 1-9. https://doi.org/10.1016/j.heliyon.2021.e07104

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CHAPTER 1

MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Apple (*Malus domestica,* Rosaceae) is a widely known fruit, which is consumed globally. The apple fruit is rich in antioxidants, flavonoids, and dietary fibre (Musacchi and Serra, 2018). The phytonutrients and antioxidants in apples may help to reduce the risk of developing cancer, hypertension, diabetes, and heart disease (Eberhardt *et al.*, 2000; Boyer and Liu, 2004; Tsao, 2015; Musacchi and Serra, 2018). However, non-optimal and inappropriate handling of apple fruit during post-harvest induce mechanical tissue injuries, microbial deterioration, increased enzymatic activity, as well as accelerated respiration rate, leading to or causing a shortened shelf life (Putnik *et al*., 2017b).

The global production of apples in 2020 was estimated to be 75.8 million tonnes, with China accounting for 57% of the total production followed by the European Union (EU) which accounted for 19% (USDA, 2020). According to USDA (2020), South Africa was ranked 4th biggest exporter of apples with an export share of 11%. Over 18 million cartons (12.5 kg each) of apples are exported by South Africa each year to the European Union (EU) (25%), Asia (19%), Africa (39%) and the Americas (3%). According to HORTGRO (2020) during the 2019/20 season, apples contributed approximately 33% which is 6.1 billion of the total 18.2 billion gross value contributed by deciduous fruits in South Africa. The main apple-growing areas are in the Eastern and Western Cape provinces of South Africa with some recent plantings of the 'Royal Gala' cultivar in the Free State province (Ntshidi *et al*., 2018). In addition, there are new low-chill apple varieties well adapted to warmer regions and required at least 800 cold units (Kriel, 2016; Meintjes, 2020).

Post-harvest losses of apples can be found all along the value chain. In particular, losses due to diseases are costly because they include the cumulative cost from growing, harvesting, to storing the product (Kitinoja & Kader, 2015). Generally, apples can be stored for up to 10 months in either cold storage, under a regular atmosphere or controlled atmosphere (CA) environment prior to marketing (Moghaddam and DeEll, 2013). The major post-harvest losses of apples occur due to fungal pathogens, mostly *Botrytis cinerea, Gloeosporium spp., Penicillium expansum* and bacterial (*Escherichia coli*) growth (Kwon *et al.,* 2018). These pathogens are major limiting factors in extending the storage and shelf life of apples. Therefore, preventing decay due to microbial contamination is the primary concern of farmers and pack-houses (Putnik *et al*., 2017a). Pre-washing in chlorinated water

or synthetic fungicide solution and maintaining appropriate optimum cold storage conditions are the most important factors to guarantee the safety, nutritional and overall quality of apples for the required sales period.

However, in recent years, growing concern about the onset of pathogenic resistance and the potential presence of toxic chlorine residues has led to a restriction in the use of these chemicals (Ferri, Yaseen, Ricelli and Colelli, 2016). In turn, this has led to an increased interest in the research for alternative control measures such as the use of thermal and non-thermal post-harvest treatments (Suslow, 2017). Conventional thermal treatments ensure microbiological safety, however, at the cost of partial loss in nutritional and sensory qualities (Lado and Yousef, 2002). On the other hand, optimal non-thermal post-harvest treatments such as electrolyzed water have the potential to slow down physiological processes, delay senescence and ripening, with improved phyto-sanitary efficacy. In addition, it maintains the nutritional quality and sensory attributes of fresh produce.

Electrolyzed water (EW) treatment is an emerging hurdle technique, which is cost-effective and environmentally friendly (Huang *et al.*, 2006). In recent years, it has been regarded as a new sanitizer and cleaner in the food industry. This is due to its simplicity of production as well as application. Electrolyzed water has been applied as a disinfectant in various fields including, agriculture, medicine, food sanitation, livestock management, and others that employ antimicrobial techniques (Rahman, Khan and Oh, 2016). It has been demonstrated that EW treatments eliminate most common types of viruses, bacteria, fungi, and spores in a relatively short period (usually within 5 to 20 s) in food products, food processing surfaces, and non-food surfaces (Olaimat and Holley, 2012; Liu *et al.*, 2013; Hricova *et al.*, 2016; Kim and Hung, 2014).

The basic setup with an electrolytic cell for the production of EW is schematically shown and annotated in Figure 1. Using electrolysis without a separation membrane, a solution with a pH close to neutral (7–8), with a low concentration of free chlorine, is obtained (Colangelo *et al.*, 2015). The solution obtained possesses special physical and chemical properties that are produced by direct current electrolysis at low voltage of salt solution, which can change its available chlorine concentration (ACC), pH value, and oxidation-reduction potential (Kim and Hung, 2014; Rahman *et al*., 2016a; Fang *et al*., 2016; Chen *et al.*, 2019a). The acidic EW contains dissolved reactive oxygen, free chlorine and is characterized by a low pH (2- 3) and a high oxidation-reduction potential (ORP> 1000 mV) (Huang *et al*., 2008). At the same time, on the cathode side, alkaline EW is produced, with high pH (10.0-11.5), highly dissolved hydrogen and low ORP (-795 to -900 mV) (Guerra Sierra *et al.*, 2019). Generally, EW is not harmful to human health as compared to chlorine, and when it comes into contact with organic matter or is diluted with tap water, it is converted back to ordinary water (Aday,

2016; Chen *et al.*, 2019a, b; Guerra Sierra *et al.*, 2019). In addition, EW is convenient to prepare, and its application cost is inexpensive (Huang et al., 2008).

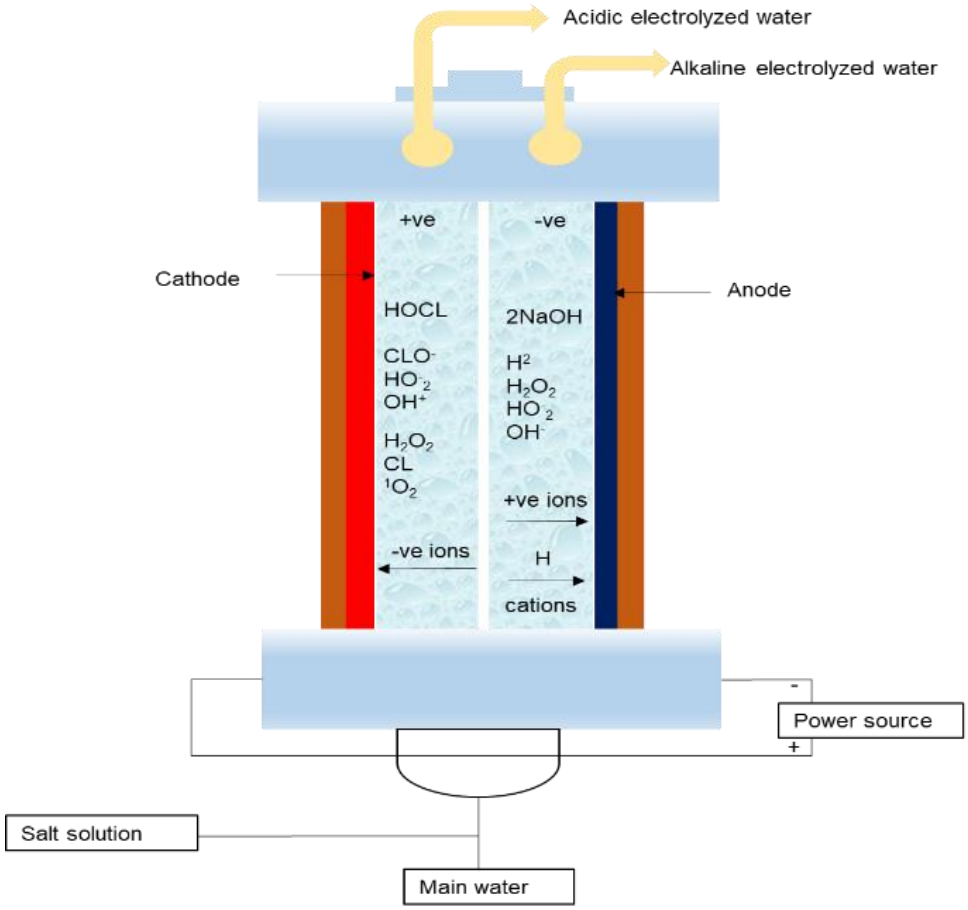

Figure 1. 1. Schematic diagram of an electrolyzed water treatment.

Over the last years, research studies have been conducted on the antimicrobial activity of EW on different food products. The post-harvest application of EW was successful in controlling microbial contaminations on citrus (Fallanaj *et al.*, 2016), strawberries (Hung *et al.*, 2010), pear (Al-Haq *et al.,* 2002), fresh-cut vegetables (Hao *et al.,* 2011). For instance, Qi, Qi *et al.*, (2018) reported that EW was effective in the removal of pesticide residues from spinach, snap beans and grapes without affecting colour and texture of the vegetable. Similarly, Ferri *et al.,* (2016) reported that treatment with the EW, improved storage quality and shelf life of apple (cv. Cripps Pink), as well as significantly reduced the concentration of some pesticide residues that were present on the apple fruit. Furthermore, Kim *et al.*, (2000) and Hung *et al.*, (2010) reported that ionized water produced using pure water combined with sodium chloride was effective in the inactivation of *Escherichia coli* on citrus fruits, strawberries and broccoli.

All these results indicate the merits of investigating the impact of EW treatments on the physiological responses, phytonutrients and overall quality of other fresh produce. This

will ensure a swift transition from chlorinated water treatment to a more eco-friendly and sustainable pre-treatment for all fresh horticultural commodities. Furthermore, to the best of our knowledge, no published work dealing with the pre-treatment practices using electrolyzed water for apples has been reported in South Africa. Therefore, this study aims to determine the impact of EW treatments on the pathological responses, physicochemical, phytonutrients and overall quality characteristics of apple (cv. Granny Smith) during storage.

1.2. Statement of the research problem

Outbreaks of foodborne diseases, the emergence of bactericide- or fungicide-resistant foodborne pathogens and stringent phytochemical residue along the value chains have heightened the demand for alternative and sustainable post-harvest treatments (Caleb *et al.,* 2013). Post-harvest phyto-sanitary measures predominantly involve the use of fungicides, which contribute to the development of bactericide or fungicide resistant pathogen populations. In turn, this results in both the use of higher doses of fungicides leading to an increase in toxic residues on fresh produce or the emergence of resistant foodborne pathogens. Hence, there is a general public resentment towards the use of synthetic preservatives and phyto-sanitary measures on fresh produce (Munhuweyi *et al.*, 2017).

Significant post-harvest losses of pome fruit of up to 70% (from single orchards) could occur sporadically in consignments shipped from South Africa to major Asian and European export destinations, due to diseases (Meitz-Hopkins, 2019). Post-harvest decay of apples during storage due to fungal pathogens such *B. cinerea* and *P. expansum* remains a challenge along the pack-houses, during retail and shelf life. Therefore, the post-harvest management of apple diseases remains a crucial engagement for the South African fruit industry throughout the value chain. Based on the limitations of the current conventional phyto-sanitary measures, alternative non-thermal and broad-based phyto-sanitary measures, which involve the use of treatments such as electrolyte water offer the potential to improve the safety of apples, while maintaining their nutritional quality attributes.

1.3. Research objectives

1.3.1. Broad objective

The broad objective of this study was to investigate the impact of electrolyzed water treatments on the physical and biochemical quality attributes, as well as changes in phytonutrients and natural microbial load of ‘Granny Smith’ apples.

1.3.2. Specific Objectives:

In order to achieve the broad objective of this research, the following specific objectives were set, which speaks to the respective chapters in this Thesis. This includes:

- To conduct an extensive literature review on the use of electrolysed water treatments (Chapter 2).
- To determine the curative efficacy of SAI-EW and AEW treatments against *B. cinerea* and *P. expansum* in ’Granny Smith’ apples during storage (Chapter 3).
- To investigate the impact of alkaline electrolysed (AIEW) treatments on phyto-compounds, antioxidant activity, physical and biochemical quality attributes and natural microbial load of ‘Granny Smith’ apples (Chapter 4).
- To characterize the change in the dynamics of culturable microbial community on the surface of ‘Granny Smith’ apples treated with AIEW (Chapter 5).

1.4. Hypotheses

Hypothesis 1:

The use of EW retains the postharvest quality and extends the shelf-life of ‘Granny Smith’ apples during postharvest storage.

Hypothesis 2:

The use of EW reduces or eliminates the presence of pathogenic and spoilage microorganisms on ‘Granny Smith’ apples.

Hypothesis 3:

The use of EW maintains the nutritional, overall quality and safety of ‘Granny Smith’ apples during storage.

1.5. Delimitations of the study

Only 'Granny Smith' apples were used in this study, no other apple cultivar was used due to the scope of the project and available resources.

1.6. Assumptions

An assumption was made that treatment of apples with EW causes the improvement of storage of apples and extends their shelf life. It was anticipated that EW treatment significantly inhibits and reduces the microbial count for fungal pathogens and spoilage microorganisms, respectively, which may be present on the fruit. It was also assumed that the implementation of EW treatment will afford the South African apple fruit industry a paradigm shift from chlorinated water towards the use of a sustainable post-harvest approach.

1.7. Significance of the research

This study investigated the impact of EW on the overall quality and safety of apples 'Granny Smith' during storage. The curative efficacy and antimicrobial efficiency of EW were demonstrated on treated apples compared to non-treated controls. It is of utmost importance to maintain the quality and microbial safety of fresh produce during the post-harvest stage. To ensure microbial safety various post-harvest handling practices are adapted, including washing of raw materials in plain/pure or chemically treated water. Washing fresh produce with pure tap water cannot be relied upon to completely remove pathogenic and other naturally occurring microflora. On the other hand, the application of EW treatments has shown the capability to reduce surface microbial load on fresh produce. Furthermore, EW treatments have been used to disinfect a variety of other products and surfaces in the food industry (Ayebah and Hung, 2005; Huang *et al.*, 2006), as well as in animal production (Mekonnen and Hoekstra, 2012). The major advantage of EW is the conversion of the reactive species generated to the final solution that is harmless to both human health and the environment.

Furthermore, EW can be produced on-site and has a less adverse impact on the environment in comparison to chlorine solution. This also eliminates the cost of transportation of the active product. The apple fruit industry and pack-houses could benefit from its quick application, disease suppression in the field, during post-harvest storage and cleaning equipment for biosecurity purposes. Hence, the South African fruit industry could also significantly minimise post-harvest losses during post-harvest handling. According to Harker, *et al.,* (2003), such measures can reduce losses from 90% to 10%. Based on literature, the use of EW treatment does not result in changes in texture, and flavour. It does not leave chlorine residues on the product surface. This could be a total game-changer for the agricultural sector and pack-houses in South Africa, as it would reduce their reliance on

fungicides and chlorine-based chemicals. It will be very cost-effective after the initial cost of the electrolysis apparatus. Its use will reduce or eliminate the cost and hazards associated with handling, transportation, and storage of concentrated chlorine solution. In turn, it will offer farmers an opportunity to improve their export markets. Besides, since the EW equipment is manufactured locally in South Africa, additional manufacturing jobs will be created, thereby, boosting the micro-economy.

1.8. Expected outcomes

The expected outcomes of this study include:

1. Provide knowledge on the possibility of maintaining the shelf life and nutritional value of apples 'Granny Smith' grown in the Western Cape region of South Africa.
2. A better understanding of the electrolyzed water treatment (EW) and its use in fruit pack houses.
3. One published manuscript or submitted for publication in a peer-reviewed, Department of Higher Education and training (DHET)-accredited journal.
4. At least one oral or poster presentation of the study data at a local or international conference.
5. A thesis towards the completion of all the requirements for obtaining the Master's degree in Food Science and Technology.

1.9. Ethical Statement

Ethical clearance with reference: 213293919/10/2020 was obtained from the Faculty of Health and Wellness Sciences at the Cape Peninsula University of Technology.

1.10. Thesis outline

The chapters in this thesis are individual entities structured in research article format. Hence, some repetitions between chapters have been unavoidable. Chapter one presents the general thesis introduction, problem statements, the aim and objectives, hypothesis, delineation and the expected outcomes at the end of the study. The comprehensive evaluation of available relevant literature is summarised in Chapter two. This chapter consists of background on apples, their post-harvest physiology, physicochemical parameters, and pathological disorders. In addition, the chapter discussed various post-harvest technologies currently employed to treat/ disinfect apple fruit in South Africa. Finally, the use of electrolyzed water treatment and its potential as an effective decontaminant in the fresh fruit industry was highlighted.

The thesis consists of three independent research chapters, which were designed to test the above-stated hypothesis linked to the study objectives. The first research chapter (Chapter 3) focused on establishing optimum treatment conditions and understanding the curative efficacy of slightly alkaline and acidic electrolyzed water (AEW) treatments. Healthy 'Granny Smith' apples were inoculated separately with *B. cinerea* and *P. expansum* and dipped in SAI-EW and AEW at varying concentrations and for different dipping duration. In chapter three, the investigation established the effective minimum EW concentration and best dipping duration for treatment of the apples. Therefore, only the most effective pre-treatment protocol was carried forward from chapter three. In chapter four, freshly harvested 'Granny Smith' apples were treated with AIEW and its impact on the overall fruit quality (TSS, TA, pH, colour, antioxidant activity, polyphenols, and total flavanol content), physiological disorder (superficial scald) and microbial load were investigated. This chapter demonstrated the efficacy and capability of AIEW to reduce microbial load and to maintain the overall quality attributes of 'Granny Smith' apples. Based on the microbial analysis conducted in chapter five, it was expedient to further characterize the microbial community isolated from the AIEW treated and non-treated 'Granny Smith' apples during storage. Thus, chapter five, which was the last research chapter focused on the identification and characterization of the major microbial community found on the apples. Finally, chapter six provided a general discussion of the entire study conducted, conclusions reached and the prospects beyond the scope of this thesis. Figure 1.2 shows the structure of the thesis.

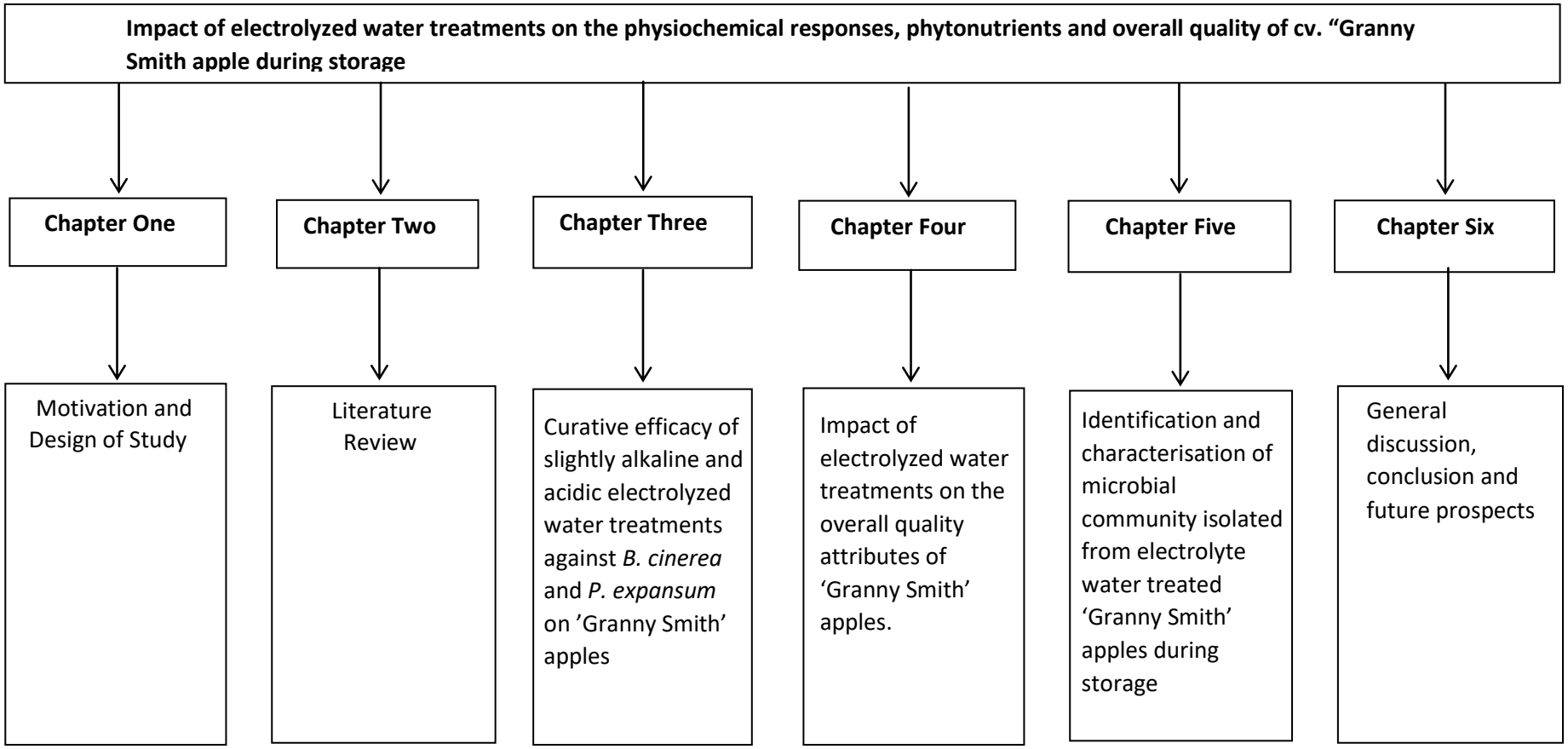

Figure 1.2. Thesis outline

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CHAPTER 2

LITERATURE REVIEW: Impact of thermal and non-thermal postharvest treatments on the disinfection of pathological and physiological disorders of apple fruit

2.1. Background

Apple (*Malus domestica*) is a climacteric fruit commercially grown in temperate regions of the world and these regions account for 50% of the world's deciduous fruit tree production (Crop *et al.*, 2013). The apple fruit belongs to the *Rosaceae* family and it is well adapted to climates where the average winter temperature is near freezing (Lauri *et al.*, 2006; Velasco *et al.*, 2010; Beltrán *et al.*, 2019). It is one of the important temperate fruits for its attractiveness, nutritional value, and raw material for many finished products (Mukhtar *et al.*, 2010; Musacchi and Serra, 2018). The fruit in its fresh state has been recognized for its attractive colour, unique taste and smell, enriched minerals, vitamins, and other health beneficial constituents (Lee *et al.*, 2003; Duda-Chodak *et al.*, 2011; Büchner, 2015; Wang *et al.*, 2015; Musacchi and Serra, 2018).

The current world apple production is estimated to be about 75. 8 million tons, with production dominated by China, producing 56.7% of the world's total apple production; followed by the European Union (USDA, 2020). South Africa is currently the leading apple producer on the African continent with about 942,203 tonnes produced in the 2019/2020 season, followed by Egypt and Kenya. Presently, approximately 24,500 ha of land is under cultivation and contributes less than 1% of the total global production of the fruit (Figure 2.1). In South Africa, the 'Golden Delicious' cultivar is the most planted variety, accounting for 23% of the total area planted, followed by the 'Royal Gala' cultivar at 17% and 'Granny Smith' at 15%. Other cultivars that have been growing steadily are the 'Pink Lady' (11%), 'Top Red' (10%), 'Fuji' (9%), and 'Cripps Red' (6%) (Figure 2.2).

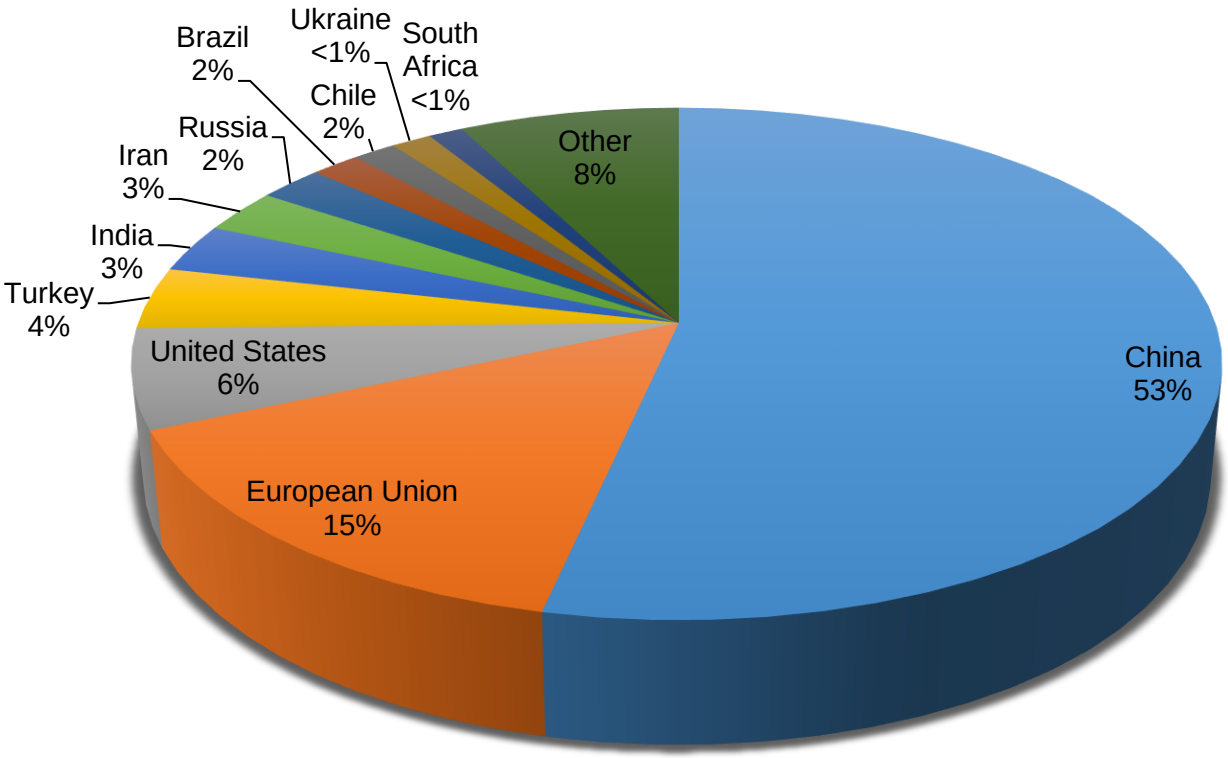

Figure 2.1. An annotated picture of global apple fruit production. Source: Statista, 2020

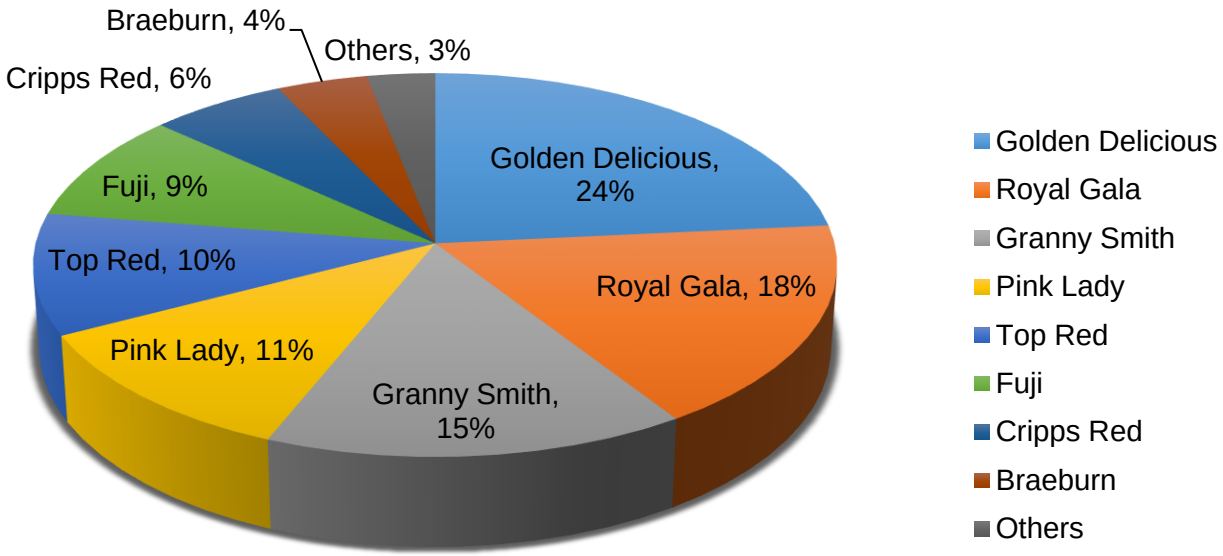

Figure 2.2. Leading apple cultivars planted (24 500 ha) in South Africa during the 2019/20 season (HORTGRO, 2020).

2.2. Export market of apple fruit produced in South Africa

The export volume of fresh apples is estimated at 570 000 tons (60.5%) of the total production (Sikuka, 2019). According to DAFF, 2019) during the 2019/20 season, apples contributed approximately 33%, which is 6.1 billion of the total 18.2 billion gross value contributed by deciduous fruits in South Africa. During 2019/2020, the number of cartons of apple fruit exported from South Africa has grown from 499,000 to 529,000 cartons, and this trend is expected to increase by 6% in the 2021 season (Rabe, 2021). In South Africa, apples account for an average export share of 66% (Ramokonyane *et al.*, 2016). The United Kingdom is the largest single-country market for South African apple exports accounting for 19% of the total exports in the 2019/20 season, followed by Malaysia (8%), Nigeria (7%), Bangladesh (5%), and the Netherlands (5%). However, Africa is the largest regional market accounting for 39% of the total South African apple exports in the 2019/20 season, followed by the

European Union (EU) at 25%, and Asia at 19% (Figure 2.3). For all the listed regions, 'Granny Smith' apple is the most demanded apple cultivar, with Australia importing only fruit from this cultivar from South Africa. Exports to Africa are largely driven by strong demand, limited competition in these markets and the fact that apples can endure suboptimal handling conditions.

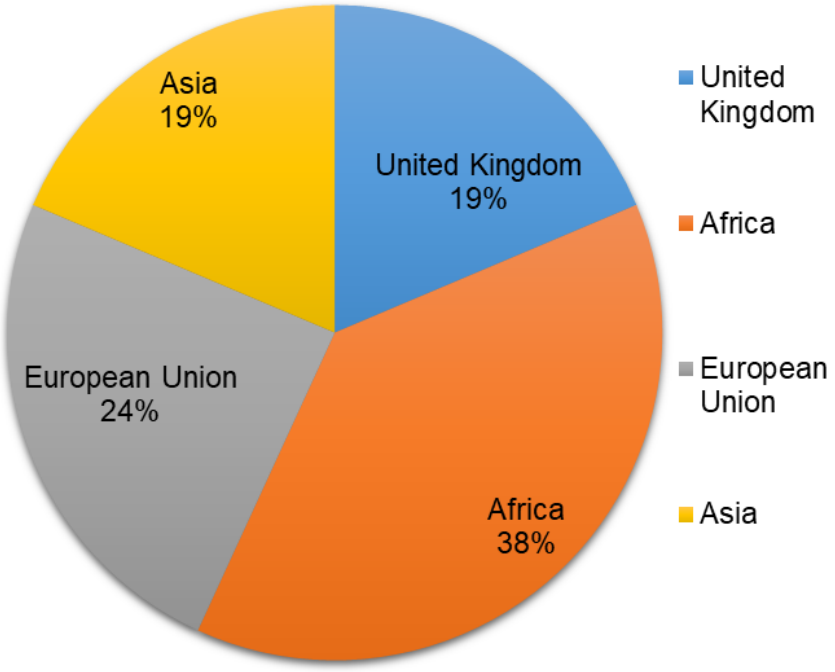

Figure 2.3. South African apple export volume per market for the 2019/20 season (DAFF and PPECB, 2019).

Securing the apple fruit industry sector for the country is important to ensure sustainable economic growth and promote the well-being of its people. However, the apple fruit industry in South Africa still contends with major post-harvest quality challenges. The main causes of these challenges are physiological (e.g., superficial scald) and pathological disorders (e.g., blue and grey mould) as well as physical injury (mechanical bruising) that occur during handling, transportation, and storage of apple fruit. Thus, these challenges require that the industry should develop and/or adopt new technologies that ensure the safety and quality of apple fruit. This will ensure that South Africa retains the market share and access to new markets for sustainability and future growth.

2.3. Nutritional/Health benefits of apple consumption

Apples represent one of the most nutritional food in a healthy diet for its contents in water (> 85.56 %), sugars (fructose > glucose > sucrose), organic acids (0.2 – 0.8 %), vitamins (mainly vitamin C, 2.3 - 31.1 mg/100 g), minerals (ash = 0.34 % - 1.23 %) and dietary fibre (Table 2.1). Some epidemiological studies have indicated that eating more apple fruits and derived products could reduce the risk of various chronic human diseases, such as diabetes, obesity, cancer, inflammation, cardiovascular diseases, and neurodegeneration (Boyer and Liu, 2004; Lu *et al.*, 2019; Wandjou *et al.*, 2020). The potential of apples in the prevention of many chronic diseases is at least in part attributed to their polyphenol constituents (Yu *et al.*, 2015; Lu *et al.*, 2019; Li *et al.*, 2020b). Five classes of phenolics are usually found in apples: flavan-3-ols / procyanidins, flavonols, phenolic acids, dihydrochalcones and anthocyanins (Telias *et al.*, 2011; Henríquez *et al.*, 2013; Tiwari and Cummins, 2013; Zupan *et al.*, 2013; Wandjou *et al.*, 2020) with the flavan-3-ols/procyanidins as the most represented class (Andre *et al.* 2012; Wandjou *et al.*, 2020). The polyphenols in apples have been shown to possess superior antioxidant activity over those in other fruits (Eberhardt *et al.*, 2000a; Lončarić *et al.*, 2020) representing 20 - 25 % of the total fruit polyphenols.

Table 2.1. Average nutrient content in apples (per 100 g fresh weight) (source: adapted from (USDA, 2020).

Constituents	Contents	Constituents	Contents
Water (g)	85.56	Pectin (g)	0.5
Energy (Kcal/kJ)	54/277	Organic fruit acids (g)	0.5
Carbohydrates (g)	13.81	Total polyphenols (mg)	111.45
Fructose	5.7	Flavanols (mg)	96.33
Glucose	0.6	Flavonols (mg)	5.66
Sucrose	0.57	Dihydrochalcones (mg)	4.18
Fibre (g)	2.7	Anthocyanins (mg)	2.15
Insoluble	0.7	Hydroxycinnamic acids	14.21
Soluble	2	Folate (mg)	9
Fat (g)	0.6	Vitamin C (mg)	12
Potassium (mg)	144	Thiamine (mg)	0.016
Calcium (mg)	10.39	Riboflavin (mg)	0.011
Magnesium (mg)	5	Vitamin B6 (mg)	0.051
Phosphorous (mg)	11		

2.3.1. Antioxidant activity

Antioxidants inhibit excess oxidative stress thereby retarding the progression of many chronic diseases (Ferretti *et al.*, 2014; Wang *et al.*, 2015). Apples, and especially apple peels, have been found to inhibit the growth of liver cancer and colon cancer cells through antioxidant activities (Wolfe *et al.*, 2003; Boyer and Liu, 2004; Tsao, 2015; Lončarić *et al.*, 2020; Nkuimi Wandjou *et al.*, 2020). The antioxidant and anti-proliferative activities of unpeeled apples are said to be greater than those of peeled apples (Duda-Chodak *et al.*, 2011). It is also known that the concentration of total phenolic compounds is much greater in the peel of apples than in the flesh, which suggests that apple peels may possess more bioactivity than the flesh (Ferretti *et al.*, 2014; Lončarić *et al.*, 2020). The total antioxidant activity of apples with the peel is approximately 83 µmol vitamin C equivalents, which means that the antioxidant activity of 100 g apples (about one serving of apple) is equivalent to about 1500 mg of vitamin C (Eberhardt *et al.*, 2000a; Boyer and Liu, 2004; Li *et al.*, 2020b). However, the amount of vitamin C in 100 g of apples is only about 12 mg (Ferretti *et al.*, 2014). Vitamin C is a powerful antioxidant, however, nearly all of the antioxidant activity from apples comes from a variety of other compounds.

2.3.2. Anti-proliferative activity

Apples have been previously shown to have anti-proliferative activity in several studies. Reagan-Shaw *et al.*, (2010) evaluated the anti-proliferative activity of apple peels against a variety of cancer cell types. The authors demonstrated that the apple peel extract (APE) obtained from organic 'Gala' apples, imparted a significant reduction in the viability of a variety of cancer cell lines. Further, their results showed a significant decrease in growth and clonogenic survival of human prostate carcinoma *CWR22Rnu1* and *DU145* cells and breast carcinoma *Mcf-7* and *Mcf-7:Her18 cells*. Also, the antiproliferative effects of APE were found to be accompanied by a G0-G1 phase arrest of prostate and breast cancer cells. Eberhardt *et al.*, (2000b) suggested that it was the unique combination of phytochemicals in the apples that were responsible for inhibiting the growth of cancer cells. Other studies have indicated that the inhibition of cancer cell growth by polyphenols and the induction of cancer cell apoptosis are caused by polyphenol oxidation rather than the antioxidant effect (Azmi *et al.*, 2005). More recently, Li *et al.*, (2020a) characterized the phytochemical profiles, antioxidant, and anti-proliferative activities of red-fleshed apples as affected by *in vitro* digestion. The authors found that the digested red-flesh or peel had a greater anti-proliferative effect on *MDA-MB-231* cells than the flesh extracts or peel extracts.

Apart from chronic disease, apples may be used to combat other prevalent human diseases. Apple phenon, a compound obtained from green apples, is composed of chlorogenic

acid, catechins, condensed catechins and procyanidins (Morinaga *et al.*, 2005; Dubreuil, 2020). The latter compound inhibited the binding of cholera toxins (CT) to Vero cells in a concentration-dependent manner. As well, toxin internalization was suppressed at 200 µg ml^{-1} of apple phenols. The mechanism most probably involves the precipitation of CT from solution or perhaps on the cell surface creating large inactive aggregates.

2.3.4. Apple phytochemicals

The phytochemical composition of apples varies between different apple varieties. There are also small changes in phytochemicals during the maturation and ripening of the fruit. Storage has been reported to have little to no effect on apple phytochemicals, but processing can greatly affect apple phytochemicals. Some of the most well-studied polyphenol compounds in apples include flavonoids such as quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside (Pavun *et al.*, 2018; Li *et al.*, 2020a). Lister *et al.*, (1994) reported quercetin glycoside concentrations of 400 - 700 mg/100 g and 250 - 550 mg/100 g in Granny Smith and Splendour apple peels, respectively, with quercetin-3-galactoside (hyperin), quercetin-3-arabinofuranoside (avicularin), quercetin-3-rhamnoside (quercetin), and quercetin 3-xyloside (reynoutrin) being the four most common.

Apples also contain flavanols ([+] catechin, [−] epicatechin) (Ferretti *et al.*, 2014; Lončarić *et al.*, 2020). Other phytochemicals are anthocyanins (cyanidin-3-galactoside), coumaric acid, chlorogenic acid, gallic acid, and certain dihydrochalcones only found in apples (Vieira *et al.*, 2011; Li *et al.*, 2020a). Apples also contain apple condensed tannins (ACT) (Zeng *et al.*, 2020). Previous studies have also evaluated the concentration of phytochemicals between the apple peels and the apple flesh. Recently, Bondonno *et al.*, (2020) examined the average concentrations of the major phenolic compounds in 91 Australian apple varieties. They found that the average phenolic concentrations among the six cultivars were: quercetin; (−)-epicatechin; procyanidin B2; phloridzin; anthocyanins; and chlorogenic acid and that all phenolic compounds except chlorogenic acid were more concentrated in the skin compared with flesh.

2.4. Physicochemical quality parameters of apple

2.4.1. Weight loss

One of the most important factors in apple post-harvest storage is the prevention of water loss and high relative humidity. Water loss of apples is due the transpiration of the fruit, which is a physiological process that persists throughout the storage period (Kiewning *et al*. 2012; Zhang *et al*. 2018; Auon *et al*. 2020). It is therefore important to minimize the rate of

ongoing metabolic events in the fruit during post-harvest storage. This is possible with the slowing of the respiration of the apple fruit (Bal *et al*., 2018). Excessive weight loss in apple fruit results in a shrivelled appearance as a result of a decrease in turgidity which renders the fruit undesirable and leads to a loss in sales. Even in the absence of visible shriveling, water loss can cause undesirable textural and flavour changes. In turn, water loss also causes additional losses in the fruit value (Bal *et al.,* 2018).

Akdemir and Bal (2020) stated that water loss causes a reduction in the weight of the fruit, leading to the deterioration of the fruit quality which results in economic loss. The highest water loss allowed in marketing apple fruit is between 5–7.5% (Akdemir and Bal, 2020). As little as 5% loss in mass can cause apples to develop a shrivelled appearance, which will render a flaccid and unappealing appearance to the fruit (Burger, 2005). Russeting is another physiological disorder of apple fruit that is influenced by water loss (Khanal *et al.,* 2013, 2019). The russeting of apple fruit fruit has been reported in literature (Noè and Eccher, 1996; Fogelman *et al.*, 2009; Knoche *et al.,* 2011), the reports have suggested that apples affected by russet shrivel first on the affected side with varying degrees of severity.

Apple fruits have a natural wax layer on the surface. The layer is a barrier to reduce water loss. Water loss from fruits is governed by the steady-state solution of Fick's first law of diffusion (Warrington, 2018). When the apple fruit temperature increases and the energy levels rise, water molecules at the fruit surface become excited. This in turn causes water molecules to share their kinetic energy in terms of thermal energy, resulting in the partial pressure difference which is one of the main driving forces behind fruit weight loss (Burger, 2005). In order to manage water loss in fruit, one needs to address: firstly, the barrier properties of the fruit, secondly, the driving force behind the water loss, and thirdly, the fruit area exposed to the atmosphere.

Korićanac *et al.* (2020) evaluated the effect of ultra-low atmosphere and normal atmosphere conditions on the quality of 'Golden delicious' and 'Indared' apples during shelf life. The authors found that the weight loss of both cultivars increased during shelf life and that the weight loss in 'Golden Delicious' apples was considerably higher in fruit stored under normal atmosphere storage conditions. In another study by Akdemir and Bal (2020), quality changes in terms of temperature and moisture was evaluated in 'Granny Smith' apple fruit in an evaporative cooling store. The authors found that the weight loss of apple fruit increased steadily during storage. Kader *et al.* (2002) reported that increasing the storage duration resulted in an increased percentage of fruit weight loss, which is in compliance with the results the authors obtained. Various strategies such as chitosan and modified atmosphere packaging (MAP) can be used to reduce weight loss of apple during post-harvest handling and storage. Plainsirichai *et al.* (2014) revealed that chitosan can maintain the weight of

apples by reducing water loss and increasing water vapour transmission. However, compared with chitosan coating, MAP is more efficient in maintaining the water content of wax apples.

2.4.2. Firmness

Another critical quality attribute influencing consumer appeal and marketing of fresh apples is its firmness (Harker *et al*., 2002; Micu *et al*., 2015; Mditshwa *et al*., 2018). Firmness has an important role on the quality, consumer acceptance and shelf life stability of apple fruit. Apple firmness along with other qualitative indicators such as sugar content, starch content, soluble solids and acidity, is an inexpensive and fast way to measure the quality of apple texture (Mollazade and Arefi, 2017). It therefore becomes important to maintain apple firmness from the orchard to the consumer, as it tends to be one of the major issues currently facing the fruit industry. Apples soften with ripening in association with cell wall breakdown prior to harvest (Pech *et al*., 2008; Payasi and Sanwal, 2010; Kader, 2013). Many studies have stated that apple softening is mediated by loss of cell-to-cell adhesion (Lurie, 1998; Johnston *et al*., 2002a; Pech *et al*., 2008; Pareek *et al*., 2011). At the cellular level, firmness depends on cell wall thickness, strength, cell size, turgor pressure and how cells bind together. This is controlled by cell shape and cell to cell adhesion (Harker *et al.*, 2010). As apples ripen, the cell wall composition changes with an increase in soluble pectin and a net loss of the non-cellulosic neutral sugars, i.e., galactose and arabinose, therefore, causing a softer fruit (Fallahi *et al.*, 1985).

Ornelas-Paz *et al*. (2020) investigated the relationship between the firmness of 'Golden Delicious' apples and the physicochemical characteristics of the fruits and their pectin during development and ripening. The authors found that the fruit firmness continuously decreased during the tested period, reaching a total firmness loss of 29.7%. Changes of fruit weight, diameter, height, tristimulus colour (L* values) as well as the content of total soluble solids and moisture in fruit correlated well with fruit firmness (r values from −0.76 to −0.97). Overall the author's results demonstrated that commercial maturity was delayed 30 days, compromising fruit firmness. Various strategies such as low temperature, calcium treatment, controlled atmosphere and ethylene inhibitors can be used to maintain the firmness of apple during post-harvest handling and storage (Tucker *et al*., 2017; Karagiannis *et al*., 2018). Calcium treatment improves the storage performance of apple fruit, and according to Watkins *et al*. (1989), apples were firmer after storage than non-treated apples. Mohebbi *et al*. (2020) investigated the Influence of early season boron spraying and postharvest calcium dip treatment on cell-wall degrading enzymes and fruit firmness in 'Starking Delicious' apple during storage. The authors found that calcium content in cortical tissue of apple fruit was significantly higher in Ca and B + Ca treatments compared to the control. After cold storage, fruit treated with Ca (Ca and B + Ca treatments) was characterized by significantly lower

ethylene production during the shelf life period, but B application alone did not affect ethylene production, which was in agreement with the results reported by Peryea and Drake (1991) for apple fruit.

2.4.3. Colour

A key quality attribute of apple fruit is its peel or skin colour, which affects consumer perception of ripeness and eating quality (Shellie and Mangan, 1994). In addition, peel colour is one of the main characteristics which enable cultivar discrimination. Apple colour changes are a signal for fruit ripening (Telias *et al.*, 2011). During ripening, apple fruit show a rapid loss of green colour, which results from the degradation of chlorophyll structures (Kader, 1999; Toivonen, 2016). The yellow to red colour of apple fruit is due to anthocyanins and carotenoids in the apple peel which becomes visible with a decline in chlorophyll (Saure, 1990). The accumulation of anthocyanin pigments in apple fruit is an important determinant of fruit quality. Anthocyanins are usually restricted to the skin of apples and provide essential cultivar differentiation for consumers. They are also implicated in the health attributes of apple fruit (Boyer and Liu, 2004).

Anthocyanins belong to the diverse group of ubiquitous secondary metabolites collectively known as flavonoids (Dar *et al.,* 2019). Flavonoids are believed to have a variety of functions including defending and protecting against light stress. Anthocyanin compounds play an important physiological role as attractants in plant or animal interactions (Harborne and Grayer, 1994; Koes *et al.,* 1994). The apple fruit cortex is usually colourless, although germplasm does exist where the cortex is highly pigmented due to the accumulation of either anthocyanins or carotenoids. Differences in colour attributes due to anthocyanins are influenced by a number of factors including the sugars and acyl side groups, and the number of hydroxyl groups on the B-ring (Harborne, 1967), the environment of the vacuole including its pH or the accumulation of specific metal ions (Brouillard, 1988), or cellular ultrastructure (Noda et al.,1994). The most common anthocyanin pigments in apple fruit are cyanidins which in the form of cyanidin-3-O-galactose are primarily responsible for the red colour in apple peel or skin (Tsao et al., 2003). There are also specific enzymes that operate in the biosynthetic pathway of apple fruit which have been previously characterized (Honda et al., 2002; Kim *et al.*, 2003).

For a given cultivar, apples with yellow colour are considered to be riper, softer and sweeter than green fruit (Telias *et al.*, 2011). Many apple cultivars are harvested according to their colour, and many consumers prefer apples of a particular colour, e.g., ‘Golden Delicious’ apples are yellow when fully ripe. Cultivars such as ‘Granny Smith’ become yellow as they ripen, and overripe fruit are characterized by their yellow background colour. Generally, the development of colour during ripening and storage depends on several cultivation and storage

factors. Colour changes are accompanied by changes in contents of acid, starch and sugar as well as fruit firmness. Thus, fruit colour may be used as an indicator of various quality parameters (Musacchi and Serra, 2018).

Low temperature plays a main role in slowing down the degradation of apple fruit quality during storage. Ganai *et al.* (2015) investigated the influence of harvest dates, precooling, calcium chloride, wax coating and storage conditions on the colour of apple 'Red delicious' by by sensory analysis. The authors found that the colour of apples degraded under ambient conditions than compared to the refrigerated samples during storage.

2.5. Post-harvest physiological and pathological disorders in apple fruit

2.5.1. Physiological disorders

Physiological disorders refer to the various forms of tissue breakdown that are not caused by pathogens or by mechanical damages (Jijakli and Lepoivre, 2006; Suzuki, 2019). Physiological disorders are mainly caused by changing environmental conditions such as temperature, moisture, and unbalanced soil nutrients, inadequate or excess of certain soil minerals, extreme soil pH and poor drainage (Singh *et al.*, 2019). They may develop as a result of the fruit response to an adverse pre-harvest and/or post-harvest environment. This is a common challenge for apple producers worldwide, which leads to remarkable losses during storage (Martins *et al.*, 2013).

Numerous studies have been carried out to explain the causes and to find remedies for these apple disorders. Most of these disorders affect discrete areas of the fruit tissue (Moor *et al.*, 2006). This could include the skin surface of the apple fruit alone (without underlying flesh tissue impact) or other internal areas of the flesh or the core region. With most disorders, the metabolic events leading to a manifestation of the symptoms are not fully understood or not understood at all (Johnson, 2008; Kader and Holcroft, 2018). Most physiological disorders are easy to identify, but others are difficult or even impossible to recognize (Singh *et al.*, 2019).

The main physiological disorders associated with apples are bitter pit, lenticels breakdown, Jonathan spot, internal breakdown, superficial scald, watercore and stem-end splitting (Rosenberger *et al.*, 2001; Jijakli and Lepoivre, 2006; Pit *et al.*, 2008; Marlow and Loescher, 2011; de Freitas *et al.*, 2015; Musacchi and Serra, 2018). These physiological disorders of apples could have a severe economic impact on the farmers' or processors' financial returns and environmental impact due to post-harvest losses. Therefore, it is crucial that these disorders are discussed further (sub-sections below) and the various possible management strategies highlighted.

2.5.1.1. Superficial scald

Superficial scald is a major physiological disorder that occurs during cold storage (below 10-15°C) of some important apple cultivars such as 'Granny Smith' and 'Red Delicious' apples (Singh *et al.*, 2019). It is characterized by brown or black patches on the fruit peel as described in Figure 2.4 (Ramokonyane *et al.*, 2016), with severity following 2-4 months of cold air storage or longer periods in controlled atmosphere (CA) storage (Gapper *et al.*, 2017). Incidence and severity are favoured by hot, dry weather before harvest, immature fruit at harvest, high nitrogen and low calcium concentrations in the apple fruit (Kader, 2013).

Figure 2.4. Superficial scald on 'Granny Smith' apple (ARC-Infruitec-Nietvoorbij, Post-harvest and Agro-processing, Stellenbosch).

Scald development is widely believed to result from the production of α-farnesene and its autoxidation to conjugated trienes (CTs) (Isidoro and Almeida, 2006; Buccheri *et al.*, 2018). The general theory is that α-farnesene, a naturally occurring volatile terpene in the apple fruit, is oxidized to a variety of products CTs. These oxidation products result in injury to the cell membranes, which eventually result in cell death in the outermost cell layers of the fruit (Fan *et al.*, 1999; Curry, 2000; Lurie and Watkins, 2012; Gapper *et al.*, 2017). The accumulation of ethylene and methyl-heptane-one (MHO) has also been arguably the cause of scald (Ju and Curry, 2002). Superficial scald symptoms start to develop within 3 to 7 days upon transferring to a temperature higher than that of the optimum cold storage (Gago *et al.*, 2015). The warm

temperatures do not cause the scald but allow symptoms to develop from the previous injury, which occurred during cold storage (Ramokonyane *et al.*, 2016). Symptoms may be visible in cold storage when the injury is severe, in this case, the symptoms intensify upon warming the fruit (Lurie and Watkins, 2012). Scald is usually not evident until after 3 months of storage.

There are a variety of post-harvest handling practices that can be used to control the appearance of superficial scald (Table 2.2). The first group of methods that proved to be effective in controlling scald were non-chemical treatments such as proper ventilation and essential oils (Scott *et al.*, 1995; Curry, 2000, 2019; Ju and Curry, 2000). In addition, controlled atmosphere (CA) storage has been widely used to control the appearance of scald in apples, however, it does not completely control scald (Hoehn *et al.*, 2009; Buccheri *et al.*, 2018; Curry, 2019). Moreover, fruit in CA storage has a high risk of off-flavours due to anaerobic respiration (Mattheis *et al.*, 2005). Other post-harvest treatments used to control scald are 1-methylcyclopropene (1-MCP) and Diphenylamine (DPA), which have been reported in the literature (Jung and Watkins, 2008; Moggia *et al.*, 2010; Sabban-Amin *et al.*, 2011; Gago *et al.*, 2015; Karagiannis *et al.*, 2018; Poirier *et al.*, 2020). However, there is a growing concern regarding the use of DPA on food materials.

Table 2.2. Summary of post-harvest control techniques reported in the literature to be effective in controlling the appearance of superficial scald in apples

Cultivar	Treatment(s)	Storage conditions	Major outcome	Reference
Granny Smith	DPA and 1-MCP	4 to 6 months at 0°C	● DPA and 1-MCP treated fruit both showed an important reduction in scald appearance compared to the control.	Moggia *et al*. (2010)
Annurca	CA and reddening	(CA: 1% O_2, 0.7% CO_2, 0.5 °C) and reddening treatment for 15 days	● CA and reddening treatment affected both, superficial scald development and fruit quality. ● The combined use of these two factors could be a good practical approach to non-chemical control.	Buccheri *et al.* (2018)
Granny Smith	DCA and initial low oxygen	0 -20 °C for 7 months	● DCA effectively controlled superficial scald on 'Granny Smith' apples for up to 7 months in cold storage. ● It offers an alternative to the use of DPA in long-term storage.	Ramokonyane *et al.* (2016)
Granny Smith	DCA	16 weeks in DCA with a 14 day interruption with regular air (RA) at 0.5 °C, 95% RH the again stored in DCA	● Maximum superficial scald incidence was 2% and 99% in repeated DCA and RA, respectively. ● Fruit stored in repeated DCA had higher fruit firmness, ground colour and titratable acidity. ● Ethylene, α-farnesene and 6-methyl 5-hepten-1 production were significantly lower in repeated DCA compared to only RA stored fruit.	Mditshwa *et al.* (2017)

DCA - Dynamic controlled atmosphere; 1-MCP - 1-methylcyclopropene

Table 2.2 Continued

Cultivar	Treatment(s)	Storage conditions	Major outcome	Reference
Golden delicious	1-MCP (smart fresh)	0.5 °C and subsequent shelf-life at room temperature, 22 °C	1-MCP treatment was effective in slowing down softening, reducing electrolyte leakage, and colour changes associated with ripening. The treatment did not affect soluble solids content and had no clear effect on fruit phenols and antioxidant activity. 1-MCP inhibited superficial scald and significantly reduced rot; however, this treatment enhanced the development of bitter pit in some orchards.	Gago *et al*. (2015)
Granny Smith	Low oxygen and 1-MCP	Cold storage, of low oxygen (LO_2, < 0.5%) atmospheres, ethanol (< 2% vapour) or (1-MCP, 0.5µLL-1) at 20 °C	Treatment was effective in reducing superficial scald in fruit following 24 weeks of cold storage.	Sabban-Amin *et al*. (2011)

DCA - Dynamic controlled atmosphere; 1-MCP - 1-methylcyclopropene

In a study by Moggia *et al.* (2010), the effects of diphenylamine (DPA) and 1-methylcyclopropene (1-MCP) on superficial scald development on cv. 'Granny Smith' was examined. The fruits were stored for 4 to 6 months at 0 °C. After 6 months, DPA- treated fruit showed similar firmness compared to the control, however, α-farnesene and CTs were lower and the cell membrane stability higher. Conversely, 1-MCP treated fruit showed a noticeable suppression in ethylene production rate and α-farnesene, along with higher firmness and lower CTs and total antioxidants. The authors concluded that the effect of 1-MCP on the investigated compounds and the reduction in scald confirmed that ethylene plays an important role in the development of scald. In another study, Mditshwa *et al.* (2017) reported on the influence of repeated dynamic controlled atmosphere (DCA) to control superficial scald on 'Granny Smith' apples. The authors demonstrated that repeated DCA treatments can effectively control superficial scald.

Both ethylene scrubbing and low-oxygen storage reduce the incidence of scald (Lurie and Watkins, 2012). The improved version of CA, DCA, is a potential replacement for chemical methods (Mditshwa *et al.*, 2018). The DCA technology has been well tested for various apple cultivars. For example, according to Ramokonyane *et al.* (2016), both DCA and initial low oxygen stress were used as alternative technologies to control superficial scald on 'Granny Smith' apple. The superficial scald symptoms only seemed to appear after transferring the fruit from a colder temperature (0 °C) to a higher temperature (warmer) (20 °C). It was therefore concluded that DCA effectively controlled superficial scald on 'Granny Smith' apples for up to 7 months in cold storage and offers an alternative to the use of DPA in long-term storage.

Hence, diverse pathways have been linked with the incidence of superficial scald further making addressing scald-related physiological responses complex. It is therefore imperative that post-harvest factors affecting its incidence and severity be understood. A better understanding of the mode of action of electrolysed water treatment in preventing the development of the disorder will assist in the development of cost-effective control strategies. Thus, further studies using integrated omics could elucidate these complexities.

2.5.2. Pathological disorders

There are more than 90 fungal species, which cause fruit rot diseases on apple fruit (Karaoglanidis and Bardas, 2006). This includes diseases such as blue mould caused by *Penicillium expansum* and grey mould caused by *Botrytis cinerea* Pers. These pathogens, along with several others such as *Alternaria, Monilinia, Mucor, Rhizopus, Fusarium, and Botryosphaeria*, have been estimated to cause up to 5 to 25% losses in apples during post-harvest storage and distribution (Okull and LaBorde, 2004a; Jijakli and Lepoivre, 2006; Karaoglanidis and Bardas, 2006; Wenneker and Thomma, 2020). Despite the technological advances in post-harvest handling of fresh fruit, losses of up to 50% occur with susceptible apple cultivars 'Braeburn', 'Golden delicious', 'Fuji', 'Granny smith', 'Red delicious', 'Honey crisp' (Jijakli and Lepoivre, 2006; Payasi and Sanwal, 2010; Paul and Pandey, 2014; Arendse *et al.*, 2018). A summary of fungal pathogens of post-harvest disease on apple fruit is presented in Table 2.3. The subsequent subsection will present information on the various pathogenic infections/diseases associated with apples, their symptoms, and management strategies.

Table 2.3. A summary of major fungal pathogens of post-harvest diseases on apple fruit.

Type of disorder	Cultivars affected	Sporulation/infection	Distinguishing symptoms	Comparison of infection and symptom development	
				Plant part	Symptoms appear
Blue Mould	'Honeycrisp', 'Royal Gala' and 'Granny Smith'	The fungus can grow at temperatures as low as -3 °C, and germination occurs at 0 °C	Wounds such as punctures, splits, bruises and limb frictions infected by *Penicillium spp.*	Fruit	
Grey Mould	'Granny Smith' and 'Golden Delicious'	The fungus favours moist, humid and environmental conditions between 9, 18.3 and 23 °C	Fluffy white to gray mycelia, gray to brown spore masses and dark to black sclerotia caused by *B. cinerea.*	Fruit	Four to 8 hours of wetness duration at optimum temperature are required for infections. Wound infections develop decay symptoms within two months after harvest whereas, stem-end and calyx-end rots symptoms are usually first seen three months or more after harvest.
Brown Rot	All apple varieties	The fungus favours temperatures of >5°C. Guentzel *et al*., (2010), found 97% RH and temperatures of 3-25°C optimum for germination	Affected fruit show a pale brown/mid brown circular rot usually associated with a wound. The rot rapidly becomes covered with buff-coloured pustules, usually in concentric rings. Although the initial infection is always through a wound, the brown rot fungus, *Monilinia fructigena*, can then spread to other fruit in a cluster by contact.	Fruit	Within five days of infection, the entire fruit can be rotted and covered with pustules. Infected fruits become mummified and tend to remain on the tree

2.5.2.1. Blue mould

Blue mould is the commonest and more destructive disease in apples during post-harvest caused by *Penicillium* (Fallik *et al.*, 2001b; Solaimani *et al.*, 2009; Vico *et al.*, 2014). Blue mould decay leads to significant economic losses during storage that also impacts fruit destined for processing by its production of the carcinogenic mycotoxins such as patulin and citrinin (Barkai-Golan and Paster, 2008). In addition, *P. expansum* infects apple fruit primarily through wounds caused by stem punctures or bruises occurring at harvest or during post-harvest handling (Vico *et al.*, 2014). The fungus can also enter the apple fruit through natural openings like via lenticels, stem ends and the calyx end (Rosenberger *et al.*, 2006).

Resistance against *P. expansum* declines quickly as the fruit matures and there is practically no resistance at harvest (Ahmadi-Afzadi *et al.*, 2013; Vilanova *et al.*, 2014). This fungus is characterized by powdery blue/ green mould (Figure 2.5). The symptoms occur as light coloured, soft lesions (Errampalli, 2014). The lesions are soft due to maceration of the tissue by polygalacturonate enzyme, which plays a significant role in *P. expansum* virulence (Jurick *et al.*, 2009). As the lesion expands the decayed portion can easily be separated from the surrounding sound tissue (Yao and Tian, 2005). The occurrence of *P. expansum* storage rot is highly variable, depending on inoculum load availability, storage conditions and mechanical damage to the fruit surface (Baert *et al.*, 2008). Accordingly, further research must be done on the control of *P. expansum*, the primary pathogen causing fungal spoilage of apples (Yu *et al.*, 2020). Post-harvest treatment of apples with fungicides has been the main treatment against this pathogen. However, with the emergence of fungicide resistance, and the increasing concern on the amounts of fungicide residues on agricultural products coupled with the potential health risks of fungicide accumulation in the food chain. Researchers have suggested the need to develop more eco-friendly methods to control both pre-harvest and post-harvest apple infection and patulin production by *P. expansum* (Rosenberger *et al.*, 2006; Caiazzo *et al.*, 2014; Errampalli, 2014).

Figure 2.5. Blue mould caused by *Penicillium expansum* on cv. 'Granny Smith' apple (ARC-Infruitec-Nietvoorbij, Post-harvest and Agro-processing, Stellenbosch).

A promising alternative to the chemical fungicides strategy is biological control. Various yeasts, bacteria and filamentous fungi have been identified and characterized for the control of blue mold caused by *P. expansum* in fruit and vegetables. Even if the modes of action of these microorganisms have not been fully elucidated, antagonistic yeasts have been selected for their capability to rapidly colonize and grow on surface wounds, thereby competing with the pathogen for nutrients and space (Errampalli, 2014; Tournas and Katsoudas, 2019; Wenneker and Thomma, 2020).

Conventionally, physical and chemical methods were applied to prevent the decay of fruits caused by *P. expansum* (Quaglia *et al.*, 2011). In recent years, there has been a general interest in the advantages of biological control over these two methods, particularly through the use of antagonistic yeasts to control post-harvest diseases (Droby *et al.*, 2016). A considerable number of yeast species such as *Pichia caribbica* have been studied and shown to control post-harvest diseases of apples. For example, Cao *et al.,* (2013) investigated the efficacy of *Pichia caribbica* in controlling post-harvest blue mold and natural decay development of apples and degrading the patulin produced by *Penicillium expansum.* The authors found that patulin production by *P*. *expansum* in apples was significantly reduced compared with the control and that *in vitro* testing

indicated that *P. caribbica* can degrade patulin directly. Furthermore, Nadai *et al.*, 2018) investigated 14 *S. bacillaris* strains to evaluate their post-harvest antifungal activity against *P. expansum* on apples. The authors demonstrated the ability of *S. bacillaris* to biologically control the apple blue mold caused by *P. expansum* without compromising product quality. Moreover, Tournas and Katsoudas, (2019) investigated the biocontrol potential of four wild yeast strains (*Meyerozyma guilliermondii* – strain YS-1, *Meyerozyma caribbica* – strain YS-3, *Cryptococcus albidus* – strain YS-4, and *Cryptococcus sp*. – strain YS-5) against *Penicillium expansum* was studied *in vivo* (on Golden Delicious apples). The test yeasts were applied to the fruits alone as well as in combination with 2% $CaCl_2$. The authors found that the addition of $CaCl_2$ to yeast suspensions facilitated much higher pathogen inhibition. At room temperature, lesion size reduction ranged between 74% and 77% during the first week. After 2 weeks of incubation, decays on yeast+$CaCl_2$-treated apples were still substantially smaller (49%-73% lower) than those on apples treated with *P. expansum* alone. At refrigeration, lesion size reduction ranged between 76% and 92% in the first month of storage and between 75% and 87% after 2 months of incubation. Decay incidence was 75% to 100% in apples stored at room temperature and 30% to 85% in those kept under refrigeration.

Morales *et al*. (2010) reviewed the effects of three control methods, the use of fungicide, yeast bio-control and controlled atmosphere, on apple decay and patulin accumulation caused by *P. expansum*. However, various studies on the chemical, biological, and physical control methods and the combination of these methods have been done (Morales *et al.*, 2007; Quaglia *et al.*, 2011; Cao *et al.*, 2013; Wenneker and Thomma, 2020; Yu *et al.*, 2020). The aggressiveness and growth of this spoilage microorganism, as well as its ability to synthesize patulin, implies that the applied control methods need to be adjusted for an effective management strategy.

2.5.2.2. Grey Mould

Botrytis cinerea is the causal agent of grey mould, a post-harvest disease associated with many fruits including apples (*Malus domestica Borkh*) (Dean *et al.*, 2012). This pathogen infects a wide range of horticultural and field crops (Munhuweyi *et al.*, 2016). Bui *et al.* (2019), reported that grey mould accounted for 28 % of the decayed apple fruit in commercial storage in Washington State alone. Although apple fruits may be infected by *B. cinerea* at harvest or earlier, grey mould usually develops during the post-harvest stage (Jijakli and Lepoivre, 2006). The infection primarily enters apple fruit through wounds and other surface defects that occur during and after harvest and in storage, creating an infection that manifests in decay (Zhao *et al.*, 2010). Infected fruit initially remains moderately firm, becoming softer with time (Aglave, 2018). *Botrytis cinerea* on fruit tends to be regular in shape, firmish, pale to mid-brown in colour, often with a darker

area around the calyx and lenticels (Figure 2.6), and sometimes as reddish spots, giving the fruit a freckled appearance (Valiuskaite *et al.*, 2006).

Figure 2.6. Grey mould caused by *Botrytis cinerea* on cv. 'Granny Smith' apple (ARC-Infruitec-Nietvoorbij, Post-harvest and Agro-processing, Stellenbosch).

To ensure that the fruits are available throughout the year, harvested apples are usually stored for extended periods (Guimin *et al.*, 2009). The storage conditions of low temperature and high humidity are usually conducive to the development of grey mould disease, ultimately resulting in considerable apple fruit economic losses. Bui *et al.* (2019), investigated the fruit of two apple cultivars – cv. 'Braeburn', which is susceptible to inoculation with *B. cinerea* and the less susceptible cv. 'Golden Delicious'. The authors investigated the interaction between the apple cultivars and *B. cinerea*, antioxidant metabolism in fruit samples from sun-exposed and shaded sides of different tissue types was measured over time. The sun-exposed tissue of cv. 'Braeburn' had higher initial levels of total vitamin C in the peel and phenolic compounds in the flesh than 'Golden Delicious', despite its greater susceptibility to grey mold. A substantial antioxidant response was recorded in diseased 'Braeburn' fruit 14 days after inoculation. This involved an elevated superoxide dismutase activity and ascorbate peroxidase activity, progressive oxidation of total vitamin C, and a decrease in peroxidase activity and phenolic content. Disease development was slower on the sun-exposed sides than on the shaded sides. Therefore, it was concluded that two cultivars appeared to utilize different strategies to defend

themselves against *B. cinerea*. The 'Golden Delicious' variety almost entirely escaped infection, and pre-harvest exposure of apple fruit to highlight / temperature stress appears to prepare them to better resist subsequent post-harvest attack and disease.

This phyto-pathogen is difficult to control because it has a broad host range, various attack modes, high survival capacity of both asexual and sexual stages in the environment (Hua *et al.*, 2018). The asexual spores of *B. cinerea* are conidia, which could be easily dispersed by wind or water, and the sexual spores of *B. cinerea* are sclerotia, which are essential for survival in an adverse environment (Brandhoff *et al.*, 2017). To date, the principal means to control grey mould rot caused by *B. cinerea* remains as the application of synthetic fungicides (Dean *et al.*, 2012). However, the fungus remains a threat to the pome fruit industry due to its ability to reproduce rapidly and survive adverse conditions for extended periods, and its potential to mutate at high frequencies to overcome fungicide treatments (Fillinger *et al.*, 2012). In addition, some plant signalling molecules, such as salicylic acid (SA), jasmonic acid (JA), can induce resistance of plants against fungal pathogens (Park *et al.*, 2007; Robert-Seilaniantz *et al.*, 2011). As a substitute for fungicides, they have been investigated and applied to control post-harvest diseases in fresh fruit with some degree of success (Terry and Joyce, 2004; Yao and Tian, 2005; Romanazzi *et al.*, 2016). For instance, Gholamnezhad, (2019) investigated SA in controlling post-harvest of apples (caused by *B. cinerea*) *in vitro* and i*n vivo*. Results indicated that the SA treatments inhibited spore germination. The application at a concentration of 25% resulted in an 89.1% reduction of disease severity compared with the untreated control.

Klein *et al.* (1997) investigated the effect of post-harvest heat treatments and calcium treatments on cv. 'Golden Delicious' apples. The apple fruits were treated after harvest with heat (air at 38 °C for 4 days or 42 °C for 1 day) or 2 % $CaCl_2$ (w/v; applied as a dip or pressure-infiltrated) or a combination of the two and stored at 0 °C for ≤ 6 months. Decay caused by *B. cinerea* after inoculation to a depth of 2 mm with a conidial suspension virtually was eliminated in stored fruit heated at 38 °C, regardless of Ca treatment. Apples punctured to a depth of 0.5 mm (but not 2 mm) and inoculated with *B. cinerea* on removal from storage were almost completely protected from post-storage decay if they had previously been pressure-infiltrated with 2% $CaCl_2$, regardless of the heat regime. Heating fruit at 42 °C and dipping in 2% $CaCl_2$ were only partially effective in preventing decay from either pre- or post-storage inoculations. In other instances, plant extracts, as well as yeast biocontrol, have been employed as post-harvest treatments to reduce the appearance of gray mould. Sernaite *et al.* (2020), investigated the antifungal activity of cinnamon, pimento, and laurel extracts *in vitro* and against postharvest gray mould on apples to determine the susceptibility of apple fruits to *B. cinerea* from different plant hosts, and to analyze the chemical composition of the extracts. The authors revealed that most

of the concentrations of the extracts that were investigated were not efficient enough when assessed in the post-harvest assay, despite having demonstrated a high in vitro antifungal effect. However, the cinnamon extract was found to be the most effective against apple gray mold; though, higher concentrations of the extracts are required for the efficient inhibition of *B. cinerea* in fruits during storage. In another study by Mewa-Ngongang *et al.* (2017), the authors investigated the post-harvest control activity of *Candida pyralidae* in controlling spoilage caused by *B. cinerea*, on apple fruit. The authors showed the potential of *C. pyralidae* as a postharvest biocontrol agent and as producer of bio-preservation compounds.

2.5.2.3. Bull's-Eye Rot

Bull's Eye rot caused by *Neofabraea spp.* is an important post-harvest disease of apple fruit worldwide (Cameldi *et al.*, 2017). Four species of this genus are known to cause this disease among which *Neofabraea vagabunda* is the main pathogen causing bull's eye rot in South Africa (Breeyan and Lennox, 2011). The symptoms usually begin on the lenticels at harvest or during cold storage (0 °C) and transportation to the market (Soto-Alvear *et al.*, 2013; Vico *et al.*, 2016). Bull's eye rot is characterized by circular necrotic lesions that are usually flat or slightly concave and generally dark and firm, causing slow and partial rotting of the apple fruit as shown in Figure 2.7 (Vico *et al.*, 2016; Pieczywek *et al.*, 2018).

Figure 2.7. Bull's eye rot originating from an infection in the stem bowl of cv. 'Golden Delicious' apple (adapted from *CL Xiao, USDA-ARS.)*

The primary infection of bull's eye rot occurs in the orchard prior to harvest and remains asymptomatic if the fruits are kept under reduced temperatures, appearing even after 3 months of post-harvest cold storage (Grove *et al.*, 1990). In addition to the fruit damage, some *Neofabraea spp.* can produce cankers in the twigs that may act as a source of primary inoculum (Grove, 1992). The current management practices to control bull's eye rot in the orchard include pruning the cankers from infected trees to minimize the buildup of inoculum during the fruit growing season; removal of the fallen fruit and dead tree branches; as well as reducing the use of irrigation systems that may otherwise promote splash dispersal of conidia from sporulation of cankers onto developing fruit (Creemers, 2014).

There are a limited number of post-harvest treatments that can be used to control the appearance of bull's eye rot in apple fruit. Fungicides registered for pome fruit can be either applied during the fruit growing season or after harvest prior to prolonged cold storage under a modified atmosphere. Aguilar *et al.* (2018) investigated the control of bull's eye rot in cv. 'Fuji' apple fruit using chemical fungicides. The post-harvest fungicides provided disease control that was far superior to other chemical compounds that were used in the study. Despite providing satisfactory control of bull's-eye rot, the integration of these three chemicals into disease management programs should proceed judiciously with consideration of their impact on the development of fungicide resistance and influence on diversity in populations of apple postharvest pathogens. In other instances, the fungicide treatments can be combined with non-chemical treatments. Cameldi *et al.* (2016) studied the influence of harvest date on the development of bull's eye rot in cv. 'Cripps Pink' and control strategies using a combination of pre-harvest fungicides and post-harvest 1-MCP. It was found that the fungicide treatments significantly reduced the bull's eye rot incidence and that when compared to the non-treated control fruit, the post-harvest treatment with 1-methylcyclopropene (1-MCP) halved the incidence of the infection.

It is therefore crucial for the South African apple producers and exporters to have alternatives for pathological disorders to safeguard the apple fruit industry by ensuring a continuous supply of quality fruit to its export markets and to provide consumers access to fresh fruit throughout the year. This has therefore led to an increased interest in the research for alternative control measures such as the use of thermal and non-thermal post-harvest treatments.

2.6. Management of post-harvest physiology and pathology of apple

2.6.1. Thermal treatments

During the past years, there has been an increased interest in the use of thermal treatments for the decontamination of apple fruit (Pankaj, 2015). Part of this interest is due

to the growing demand to decrease the post-harvest use of chemicals against pathogens and insects (Geysen *et al.*, 2005). There are three post-harvest thermal methods currently in use to treat fruit commodities; hot water, vapor heat and hot air (Klein and Lurie, 1991; Lurie, 1998; Lurie *et al.*, 1998). Hot water was originally used for fungal control but has been extended to the disinfestation of insects (Jemric *et al.*, 2006; Lu *et al.*, 2007; Poirier *et al.*, 2020). Vapor heat was developed specifically for insect control, and hot air has been used for both fungal and insect control and to study the response of commodities to high temperature (Lurie, 1998; Fallik, 2004; Kader, 2013).

The heat from the thermal post-harvest treatments can inhibit pathogens by slowing down germ tube elongation, inactivating or killing germinating spores outright, and reducing disease symptoms (Liu *et al.*, 2012). Heat treatment can also prevent storage decay by simulating the host defense responses in fruit tissue (Romanazzi *et al.*, 2016). However, severe heat could lead to undesirable effects, such as a change in colour, texture and loss of nutrients, thus, motivating researchers to explore non-thermal alternatives (Wand *et al.*, 2006; Lu *et al.*, 2007; Maxin, 2012; Kader, 2013; Caleb *et al.*, 2016; Poirier *et al.*, 2020). Hence, the thermal treatment of fresh fruit and vegetables must be optimized for each specific commodity.

2.6.1.1. Hot water dip*/* spray

During cold storage, apple fruit may develop a variety of diseases caused by fungi such as blue mould (*P. expansum*), grey mould (*B. cinerea*), and bull's eye rot (*Neofabraea spp.*) (Nunes *et al.*, 2001; Børve *et al.*, 2013; Grantina-Ievina, 2016). Among alternative methods to control fruit post-harvest diseases, heat applied by fruit dipping in hot water appears to be one of the most promising methods (Lurie *et al.*, 1998; Klein and Lurie, 2018). Hot water treatment (HWT) is an effective physical treatment, free of chemical residues, and readily applicable in the fruit industry during the washing process (Aguayo *et al.*, 2015). It is one of the most easily applied and environmentally safe (Drake *et al.*, 2005; Geysen *et al.*, 2005). The heat from the hot water can inhibit the pathogen by slowing down the germ tube elongation, inactivating or killing germinating spores outright, and reducing disease symptoms (Liu *et al.*, 2012). However, heat damage to the tissues of sensitive fruit can be caused if the temperatures are too high or if the duration of the treatment is too long (Erkan *et al.*, 2005). According to Gasser *et al.* (2015), the threshold temperatures of the hot water treatments for apple fruit are in the range of 50 to 52 °C for 150 s.

Several data have shown the efficacy of HWT applied to apple fruit against *M. fructigena, Gloeosporium spp, P. expansum, Neonectria galligena* and *B. cinerea* (Fallik *et al.*, 2001a; Maxin *et al.*, 2012). For example, Maxin *et al.* (2012a) investigated the suitable HWT to prevent decay incidence and maintain fruit quality of cv. "Ingrid Mariie"

apple fruit against *Gloeosporium rot, N. galligena* and *M. fructigena.* The apple fruits were subjected to different ranges of temperatures (49°C, 51°C, 53°C) and dipping periods (60 s, 120 s and 180 s). The authors demonstrated that *M. fructigena* was reduced up to 83% by HWT at 53 °C and 180 s dipping time. With this treatment, *Gloeosporium* rot was reduced by up to 92%, but the incidence of decay with *N. galligena* increased with the 60 s dipping duration. Furthermore, scale symptoms were found when the fruits were treated at 51 °C. Therefore, treatment with 53 °C and 180 s was recommended and for cultivars with high sensitivity to skin disorders, dipping for 120 s was recommended. In another study by Maxin *et al.* (2012b), it was demonstrated that the apples were resistant to green mold when the fruits were wound-inoculated after the heat treatment, indicating a potential defense-induced response to the pathogen infection in treated apples.

Furthermore, Poirier *et al.* (2020) investigated the treatment of 'Granny Smith' apples with hot water and 1-MCP at harvest or following long-term (> 3 months) in-house and commercial ULO-CA for two consecutive years. The authors found that the scald induction was effectively delayed during ULO-CA storage and resumed upon return to air storage and that 1-MCP and hot water treatments applied after four months of ULO-CA storage were equally effective at controlling scald during the subsequent air storage as treatments applied at harvest. In another study by Nyamende *et al.,* (2021), the effects of HW treatment (45°C) under varying dipping durations (5, 10 and 15 min) on physicochemical quality of apple were investigated. The authors found that HW treatment duration (15 min) had beneficial effects on flesh firmness, fruit colour, total soluble solid (TSS) and titritable acidity (TA) by the end of the storage.

HWT has been successfully applied on other fruit types to control postharvest pathogens such as *Monilinia spp.* on stone fruit (Casals *et al.*, 2010), *C. gloesporioides* on mangos (Alvindia and Acda, 2015), *C. musae* on bananas (Alvindia, 2012), *B. cinerea* on tomatoes (Fallik *et al.*, 2002) and *Penicillium spp.* on citrus fruit and apple (Nunes *et al.*, 2001).

2.6.1.2. Vapour / moist air

Vapour / moist air is a method of heating fruit with air saturated with water vapour at temperatures of 40–50 °C to kill insect eggs and larvae as a quarantine treatment before fresh market shipment (Miller and McDonald, 1997). It consists of stacking the boxes of fruits in a room that is heated and humidified by the injection of steam. Heat transfer is by condensation of water vapour on the cooler fruit surface (Zhou *et al.*, 2002; Lydakis and Aked, 2003). In modern facilities, the vapour heat includes forced air which circulates through the pallets and heats the commodity more quickly than vapour heat without forced air (Gaffney and Armstrong, 2015).

Limited studies have been conducted for using vapour or moist forced air to

disinfect apple fruit (Shellie and Mangan, 2013; Erkan, Pekmezci and Wang, 2005; Patil *et al.*, 2019). In a study conducted by Neven *et al.* (1996), apples were exposed to forced hot air to generate simulated fruit heating profiles. Three apples varieties were exposed to three different temperatures (44, 46, and 48 °C) of forced air under either moist or vapour conditions. The fruit heating profiles were used to program a computer-controlled water bath to simulate fruit heating for treatment of codling moth. Heat treatments in which fruit core temperatures did not exceed 43 °C were the least effective in causing mortality, whereas treatments that produced core temperatures 46 °C were the most effective. It was found that heating rates were slower for the moist forced air heating than vapour forced air; these rates resulted in a slightly less effective larval kill. Mortalities of larvae infesting apples exposed to vapour forced air at 44 °C approximated those obtained in the computerized water bath system. Whenever the heat treatment was followed by cold storage, larval mortality was enhanced.

The effectiveness of vapour heat treatment was confirmed for other horticultural commodities. For example, Aharoni *et al.* (2010) reported that post-harvest vapour heat treatment at 38 °C for 8 h applied to afternoon or evening harvested basil markedly reduced decay susceptibility. Similarly, Singh and Saini (2014) determined the influence of vapour heat treatment on the qualitative and quantitative measurement of aroma volatiles during ripening in mango fruit (cv. Chausa). The vapour heat treatment at 48 °C for 20 min accelerated the process of fruit ripening and led to the edible soft stage within 4 days, after heat treatment against 8 days in control.

With growing interest in incorporating post-harvest vapour heat treatments to improve the quality and safety of apple fruit, there still needs to be more research to investigate their potential. Therefore, these treatments must be carefully investigated to determine the optimal range of barrier, mechanical and antimicrobial properties.

2.6.1.3. Hot air

Hot air is another form of heat treatment used in a variety of fruit to maintain post-harvest quality during storage and offers an alternative to synthetic chemical fungicides (Lurie, 1998). It can be applied by placing fruit in a heated chamber with a ventilating fan, or by applying forced hot air where the speed of air circulation is precisely controlled (Geysen *et al.*, 2005). Hot air, whether forced or not, heats more slowly than hot water immersion or forced vapour heat, although forced hot air will heat produce faster than a regular heating chamber (Lurie, 1998; Barkai-Golan., 2001). Forced hot air, however, has been used to develop quarantine procedures (Gaffney and Armstrong, 2015). One reason is that the high humidity in vapour heat can sometimes damage the fruit being treated, while the slower heating time and lower humidity of forced hot air can cause less damage (Geysen *et al.*, 2005).

In apple fruit, hot air treatment at 38 °C for 4 days has been considered to be the optimal temperature and duration to preserve post-harvest storage quality (Lurie, 1998; Lu *et al.*, 2007). This treatment has been proven to delay ripening and maintain the firmness of apple fruit to improve consumer acceptability while also controlling the development of common post-harvest diseases caused by *P.* expansum, *B. cinerea* and *C. acutatum* (Klein and Lurie, 1991, 2018; Shao *et al.*, 2007) Hot air induces increased residual activity and enhances the healing process. However, the negative impacts of HA include enhanced yellowing of peel and reduced titratable acidity (TA) and weight in apples. These three factors are regarded as the signals of senescence for fruit. Dentener *et al.* (1996) investigated the mortality response of light brown apple moth (LBAM) and long-tailed mealybug (LMB) on persimmons to hot air heat treatments at air temperatures between 44 °C and 50 °C. LMBs were more tolerant to heat treatment than LBAM and there was no difference in mortality for both species between insects found on the surface of the fruit, or under the calyx. An estimated treatment time of 12.4 h at 44 °C was needed to achieve 99% mortality of LMB. This time decreased with increasing temperature to about 3.8 h at 50 °C. Further investigations should confirm hot air treatment of persimmons as a viable disinfestation method against LMB and LBAM, causing only minor levels of heat injury to the fruit but capable of inducing additional chilling resistance.

Very limited studies have been conducted on the hot air postharvest treatment of apple fruit. For example, Neven et al. (1996) exposed apples to forced hot air to generate simulated heating profiles. Heat treatments in which fruit core temperatures did not exceed 43 °C were the least effective in causing mortality, whereas treatments that produced core temperatures ≥ 46 °C were the most effective. Heating rates were slower for the moist forced air heating than vapour forced air; these rates resulted in a slightly less effective larval kill. Mortalities of larvae infesting apples exposed to vapour forced air at 44°C approximated those obtained in the computerized water bath system. Whenever the heat treatment was followed by cold storage, larval mortality was enhanced. In other studies using hot air treatment, Wang et al. (2010) showed that hot air (HA) reduced green mold decay in Chinese bayberries through enhancing defense-related enzyme activities. Furthermore, Jin *et al.* (2016) investigated the effect of hot air (HA, 45°C, 3.5 h) treatment on reducing gray mold caused by *B. cinerea* in strawberry fruit and the possible mechanisms. The results showed that HA treatment significantly reduced lesion diameter and enhanced activities of chitinase (CHI), β-1, 3-glucanase and phenylalanine ammonia-lyase (PAL) in strawberry fruit.

A high temperature forced air quarantine treatment to kill Mediterranean fruit fly, melon fly and oriental fruit fly on papayas was developed by Armstrong *et al.* (2015). The four-stage treatment forced 43 ± 1, 45 ± 1, 46.5 ± 1, and 49 ± 0.5 °C hot air over the papaya surfaces until the fruit centre temperatures at the end of each temperature stage

reached 41 ± 1.5, 44 ± 1, 46.5 ± 0.75, and 47.2 °C, respectively. Relative humidity of 40-60% during treatment prevented fruit damage. When the fruit centre temperatures reached 47.2°C, the papayas were immediately hydro-cooled until the fruit centre temperatures were ≤30°C. Phyto- toxicity tests showed that the HTFA treatment was not detrimental to fruit quality. Survival tests with the HTFA treatment until final fruit centre temperatures were 43.2, 45.2, or 46.2 °C showed little or no survival between 46.2 and 47.2 °C for *C. capitata*, and between 45.2 and 46.2 °C for *D. cucurbitae* and *D. dorsalis.* It was observed that *D. cucurbitae* was more susceptible to the HTFA treatment than *C. capitata* or *D. dorsalis*. Survival tests also showed that either first or third instars were more susceptible to the HTFA treatment than eggs for all three fruit fly species.

2.6.2. Non-thermal treatments

2.6.2.1. Cold Plasma (CP)

Cold plasma (CP) is defined in terms of partly ionized gas in which the overall temperature is quite low because the stored energy is mostly in free electrons (Phan *et al.*, 2017). It involves exposing food to this ionizing radiation including charged particles, electric fields, photons, or reactive species to disinfect microbes and pesticides while ensuring the safety of many fruits (Niemira, 2012a). In the past, this technology was used for sterilization of sensitive materials and now it is extended in the food industry as a novel technology (Rezaei *et al.*, 2019). Cold plasma provides high efficiency on microbial inactivation as well as removing toxicant residues at low temperature (< 50 °C), resulting in improved shelf life and safety of foods and reduced usage of preservatives. It is also compatible with most packaging types and modified atmospheres due to *in situ* generation of the reactive species.

The postharvest application of cold plasma on apple fruit has been extensively studied (Table 2.4). For instance, Baier *et al.* (2014) found that there was a complete reduction of *E.coli and L. innocua* on apples exposed to cold plasma for 20 seconds. The authors further stated that the inactivation was mainly due to the smooth surface of apples, which allowed for more exposure. In another study, Bhide *et al.* (2017), found that *E. aerogenes* reduced by 1.86 ± 1.27 log CFU/63.64 cm^2 after 492 s of exposure to cold plasma on cv. 'Golden Delicious' apples. Segura-Ponce *et al.* (2018), reported the effect of low-pressure cold plasma (LPCP) on the inactivation of *E. coli* and *L. innocua* on fresh-cut apple skin and its influence on wettability. The authors demonstrated that LPCP treatments using Ar, O_2, or Ar-O_2 mixture for 20 min were the most effective to inactivate *E. coli* with O_2, while the LPCP treatment with N2 for 20 min reduced *L. innocua* the most for ($p < 0.05$). The highest increase in surface wettability was observed in samples treated for 20 min with O_2 and Ar-O_2. Different LPCP treatments had no great effectivity on the

inactivation of *E. coli* and *L. innocua* on fresh-cut apple surfaces, but all treatments changed the surface wettability of apples, making it more hydrophilic. This can be considered as a negative effect of the LPCP treatment because it can facilitate the adhesion and proliferation of re-contaminating microorganisms.

Even though, the majority of studies on the application of CP focused on bacterial decontamination, few studies demonstrated the potential of CP for enzyme inactivation. Tappi *et al.* (2014; 2019) studied the effects of cold plasma using a dielectric barrier discharge equipment on some quality parameters of different apple (cvs. Pink Lady, Fuji, Red Delicious and Modi), with a focus on polyphenol oxidation inhibition. In terms of browning, a significant decrease about 65% was observed in treated samples (for 30 min and after 4 h of storage) compared to the. Polyphenol oxidation residual activity decreased linearly by increasing the treatment time (up to about 42%). In general, the authors found that the treatment appeared to slow down the metabolic activity of the tissue. Other qualitative parameters were only slightly affected by the treatment. Ramazzina *et al.* (2016) found that cold plasma treatment caused a slight reduction in antioxidant content and antioxidant capacity of fresh cut 'Pink Lady' apples. On the other hand, Bußler *et al.* (2017) studied the effect of cold plasma using a microwave-driven plasma torch equipment on polyphenol oxidase and peroxidase inhibition of apple fruits (cv). The study showed that plasma air processing is capable of reducing the activity of polyphenol oxidase and peroxidase in the freshly cut tissue of apples. Following exposure to cold plasma for 10 min, the polyphenol oxidase activity was reduced by about 62% in fresh-cut apple.

Table 2.4. Summary of effects of cold plasma (CP) processing on the quality of apple fruit.

Food matrix	Plasma source	Initial microflora	Treatment conditions	Quality observation	Microbial observation	Reference
Golden Delicious apples	Gliding arc plasma (60 Hz, 14 Kv)	*E. coli* O157:H7 and *Salmonella* Stanley (10^8 CFU/mL)	Flow rate: 10, 20, 30 and L/min Time: 1-3 min Temperature: 41 °C and 51 °C	N/A	• Microbial inactivation for *Salmonella* Stanley was time dependent at all flow rates and *E. coli* was time dependent at 10,20,and 30 L/min but similar for all time intervals at 40 L/min. • Significant ($P < 0.05$) reductions from the untreated control, with 40 L/min more effective than were lower flow rates. • The best reduction of *E.coli* ranged between 3.4 to 3.6 log CFU/mL. • The best reduction of *Salmonella* Stanley ranged between 3.1- 4 log CFU/mL.	Niemira & Sites, 2008)

Table 2.4. Continued

Food matrix	Plasma source	Initial microflora	Treatment conditions	Quality observation	Microbial observation	Reference
Apples	Dielectric barrier discharge (DBD) generator (20kHz, 10 Kv)	*E. coli* and *L. innocua* (10^8 CFU/mL)	Samples placed at fixed distance of 17 mm Time: 20, 40, 60 and 90 s Temperature: 23°C	Smooth surface of the apples	• Almost complete reduction of *E.coli* and *L. innocua* within the first 20 s • 4.7 log reduction after 60 s • Higher inactivation could be sue to the smooth surface of the apples.	(Baier *et al.*, 2014)
Fresh cut 'Pink Lady' apples	Dielectric Barrier Discharge (DBD) generator (12.7 kHz, 19 V)	N/A	Samples placed at a linear distance of 2 mm Time: 10, 20 and 30min Temperature: 22 °C	• In terms of browned areas, a significant decrease was observed in treated samples compared to the control (up to about 65% for 30 min and after 4h of storage). • In general the treatment appeared to slow down the metabolic activity of the tissue.	N/A	(Tappi *et al.*, 2014)

Table 2.4. Continued

Food matrix	Plasma source	Initial microflora	Treatment conditions	Quality observation	Microbial observation	Reference
Apple peel and pulp	Microwave discharge generator (2.45 GHz, 1.1 kW)	*E. coli, P.marginalis, P. carotovorum, L. innocua, S. aureus, B. atrophaeus* Namakura, and *C. albicans* (10^8 CFU/mL)	Fixed distance: 25 cm Time: 7s Gas Temperature: 4000K Post-treatment: 5, 10 and 15 min	N/A	• 6.2 log reduction observed on the apple peel. • Apple pulp showed lower antimicrobial effect for post plasma storage • 10 or 15 min did not show an increase in the antimicrobial effect. • *E. coli, P.marginalis,* and *P. carotovorum* reduced to detection level after 5 min of post- treatment. • *L. innocua* and *S. aureus* had a reduction of 2.3, 1.8, 3.2 and 3.4, 6.2, 4.6 log steps respectively after 5, 10 and 15 min. • *B. atrophaeus* Namakura, and yeast *C. albicans* reduced by 1.4 to 3 log steps after 15 min post treatment.	(Schnabel *et al.*, 2015)

Table 2.4. Continued

Food matrix	Plasma source	Initial microflora	Treatment conditions	Quality observation	Microbial observation	Reference
Fresh cut 'Pink Lady' apples	Dielectric barrier discharge gas plasma generator (12.7 kHz, 150 W.	N/A	Samples placed at a distance of 70 mm Time: 120 (60 + 60) min Temperature: 22 °C	• Plasma treatment caused only a slight reduction of antioxidant content (10 %) and antioxidant capacity, mainly limited to the amphiphilic fraction. • Polyphenol extract obtained from plasma-treated samples does not induce significant changes in cell viability of human cultured colonocytes, in comparison with extracts obtained from untreated apples slices.	N/A	(Ramazzina *et al.*, 2016)
Golden Delicious apple peel	Golden Delicious apple peel	*E. aerogenes* (6.81 log CFU/63.64 cm^2)	Samples placed at a fixed distance of 7.7 cm Time: 492 s	N/A	• No reduction observed on the microbial reduction for control samples. • *E. aerogenes* reduced by 1.86 ± 1.27 log CFU/63.64 cm^2 after 492 s.	(Bhide *et al.*, 2017)

Table 2.4. Continued

Food matrix	Plasma source	Initial microflora	Treatment conditions	Quality observation	Microbial observation	Reference
Fresh cut 'Granny Smith' apple slices	Microwave-driven discharge used to generate plasma processed air (PPA) (2.45 GHz, 1.2 kW)	N/A	Flow rate: 20 L min^{-1} Time: 2.5, 5,7 and 10 min Temperature: 22 °C	• PPA processing was capable of reducing the activity of PPO and POD in the freshly cut tissue of apple. • Following exposure to PPA for 10 min the PPO activity was reduced by about 62% in fresh cut apple. • POD, as the more temperature-stable enzyme, was even less stable upon PPA treatment for 10 min and was reduced by about 65% in fresh cut apple tissue. • Browning different from the habitual nature of enzymatic browning occurred upon exposure of the apple tissue to PPA. • The pH value on the tissue surface dropped to 1.5 while cell integrity and dry matter content were not significantly affected.	N/A	(Bußler *et al.*, 2017)

Table 2.4. Continued

Food matrix	Plasma source	Initial microflora	Treatment conditions	Quality observation	Microbial observation	Reference
Golden Delicious apples	Indirect corona discharge system (200 W, 50Hz)	*Salmonella Typhimurium*; *Salmonella Choleraesuis* and *Escherichia coli* (10^8 CFU/mL for each bacteria)	Samples exposed at a fixed distance of 35 mm with an input power of 200 W. Time: 30, 60, 120, 180, and 240 s.	N/A	• Highest reduction observed for *Salmonella* (5.3 log10 CFU/cm^2). • The best reduction for *E. coli* was 5.5 log10 CFU/cm^2.	(Kilonzo-Nthenge et al. 2018b)
Fresh cut 'Pink Lady', 'Fuji', 'Red Delicious' and 'Modi' apples	Dielectric barrier discharge (150W)	N/A	Samples placed at a fixed distance of 9cm. Time: 30 and 60 min Temperature: 22 °C	• Noticeable reduction of superficial browning was observed in all cultivars. • Textural parameters were affected by plasma treatments only in Red Delicious apples.	N/A	(Tappi *et al.*, 2019)

Table 2.4. Continued

Food matrix	Plasma source	Initial microflora	Treatment conditions	Quality observation	Microbial observation	Reference
Granny Smith apple peel	Dielectric barrier discharge generator (17 kV)	*S.Typhimurium* and *L. innocua* (10^8 CFU/mL)	Samples placed at a fixed distance of 9 mm Time: 5, 8, 10 s Flow rate of H2O2: 5 mL/min Air pressure: 7psi	• Thetreatments had no significant difference in the colour and texture of apples corresponding controls for apples during 14 days of shelf-life at 17 °C. • The pH of apples did not exhibit obvious changes and the soluble solids content of the apples were relatively stable.	• The inoculated bacteria were significantly reduced to a level below the detection limit (0.70 log CFU/piece) on the smooth surfaces of apples.	(Song *et al.*, 2020)
Fresh apple 'Gala' cubes	Glow discharge (80 kV)	N/A	<u>Glow Discharge:</u> Flow rate: 10, 20, and 30 mL/min Time: 10, 20, and 30 min	• Changes in sugars and organic acids were observed after the application of both plasma technologies. • Glow discharge plasma application induced a decrease in sucrose content and the increase in glucose, fructose, and malic acid.	N/A	(Farias *et al.*, 2021)

2.8.2.2. Pulsed electric field (PEF)

Pulsed electric field (PEF) is a non-thermal food preservation method that uses short pulses of electricity for microbial inactivation and causes minimal detrimental effects on food quality attributes (Raso and Heinz, 2006). Electrical fields in the range of 5-50 kV /cm generated by the application of short pulses of high voltage (μs) between two electrodes cause microbial inactivation at temperatures below those used in thermal processing (Odriozola-Serrano *et al.*, 2013; Jin *et al.*, 2014, 2017; Yang *et al.*, 2016). The precise mechanisms by which microorganisms are inactivated by pulsed electric fields are not well understood; however, it is generally accepted that PEF leads to the sieving of microbial membranes (Timmermans *et al.*, 2019). Numerous studies have demonstrated the ability of PEF to obtain shelf-stable plant-based liquid foods with high nutritional and sensory value (Odriozola-Serrano *et al.*, 2013; Saldaña *et al.*, 2014). Most of these studies have been focused on liquid foods, such as juices and beverages. There is an increasing interest in PEF pre-treatment of fresh produce products, such as apple fruit (Angersbach *et al.*, 2000; Bazhal *et al.*, 2001; Lebovka *et al.*, 2004).

Furthermore, PEF has been studied for killing pathogenic microorganisms such as *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* as well as spoilage bacteria, moulds and yeasts (Jin *et al.*, 2009a,b, 2014, 2015, 2017; Gurtler *et al.*, 2011; Jin, 2017). Depending on the electric conditions, such as electric field strength and the number of pulses, PEF treatment can provide different effects and can be used for various applications (Yu *et al.*, 2015). PEF treatments have exhibited the potential of maintaining the physio-chemical quality of liquid food products, without substantially impacting the sensory qualities and health-related compound makeup (Guo *et al.*, 2014). Some of the most important current advantages and technical drawbacks or limitations of the PEF technology are listed in Table 2.5.

Table 2.5. Advantages and limitations of PEF technology.

	Advantages	Disadvantages
PULSED ELECTRIC FIELD (PEF)	● Kills vegetative cells; ● Colours, flavours and nutrients are preserved; ● No evidence of toxicity; relatively short treatment time; ● Accelerated thawing; ● Decontamination of heat-sensitive foods; ● Best suitable for liquid foods; ● Pasteurization of fruit juices, soups, liquid egg and milk; ● No environmental hazard.	● High initial cost; no effect on enzymes and spores, ● PEF alone; only suitable for liquids particles in liquids; ● Products of electrolysis may adversely affect foods; energy efficiency not yet certain; ● The presence of bubbles, which may lead to non-uniform treatment as well as operational and safety problems; ● Limited application, which is restricted to food products that can withstand high electric fields; ● The particle size of the liquid food in both static and flow treatment modes ● The maximum particle size in the liquid must be smaller than the gap of the treatment region in the chamber in order to maintain a proper processing operation.

Sources: Grahl and Märkl. (1996); Charles-Rodríguez *et al.* (2007); Toepfl *et al.* (2007); Donsì *et al.* (2010); Vico *et al.* (2014); Yang *et al.* (2016); Zeng *et al.* (2016); Soliva-Fortuny *et al.* (2017a); Yu *et al.*(2017)

The effects of pulsed electric fields (PEF) on the total phenolic, flavonoid and flavan-3-ol contents, as well as on the antioxidant capacity of apple (cv. Golden Delicious) stored at different temperatures (4 and 22 °C) along 48 h were studied by Soliva-Fortuny *et al.* (2017). Contents of phenolic compounds observed in PEF-treated apples were higher than those of untreated apples. The mildest PEF treatment (0.008 kJ kg^{-1}) produced the maximum increases of total phenolic (13%) and flavan-3-ol (92%) contents in apples stored during 24 h at 22 °C, while it was observed at 4 °C for flavonoids (58%). On the other hand, the antioxidant capacity of apples was enhanced by 43% compared to that of untreated with the mildest PEF treatment after 12 h at 4 °C and by 15% after 24 h at 22 °C. Most of the work done has been on apple juice. There is currently very limited information about the use of PEF technology in apples, and thus, its application needs to be explored.

2.8.2.3. Ozone

Ozone is a strong, naturally occurring oxidizing agent that has found wide application in the disinfection of fruit (Pandiselvam *et al.*, 2020). Previously, it has been suggested that ozone can be used as an alternative sanitizer to replace the traditional chlorine (Lv *et al.*, 2019). The advantages of ozone include a more effective function against a wide range of microorganisms and the fact that it does not result in any chemical residues as compared to traditional chlorine. Recent studies have shown that ozone can reduce the postharvest decay of many fruits by inhibiting pathogens or by inducing decay resistance (Alexandre *et al.*, 2012; Coelho *et al.*, 2015; Antos *et al.*, 2018; Zhou *et al.*, 2019). However, ozone is a highly reactive compound that might cause physiological changes in crops, including tissue damage, induction of defence mechanisms, induced ethylene production and increased respiration (Liu *et al.*, 2016; Karagiannis *et al.*, 2020). Although the effect of ozone on fruit varies depending on the type of fruit, ozone concentration and duration of exposure, the application of ozone is beneficial in maintaining the quality and increasing the shelf life of fruits (Shynkaryk *et al.*, 2015; Pandiselvam *et al.*, 2020).

The use of ozone as a postharvest treatment on apple fruit has been widely studied. For example, Yaseen (2015) found that ozone was suitable to control and reduce losses caused by *P. expansum* during postharvest storage. In another study by Antos *et al.* (2018), the potential of ozone-enriched atmosphere for the improvement of the shelf life of apples during cold storage was investigated. The results showed that the exposure to ozone was unsuccessful in terms of inhibition of fungal disease development. On the other hand, the tests of the physical properties showed that utilization of ozone slowed down the ripening of the apple fruit. Therefore, extending their shelf life provided that they

were not infected. Furthermore, Sheng *et al.* (2018) investigated *L. innocua* on 'Fuji' apples fruit surfaces during commercial cold storage with and without continuous low doses of gaseous ozone. Un-waxed 'Fuji' apples of commercially acceptable maturity were inoculated with 6 - 7 Log_{10} CFU of *L. innocua* per apple, and subjected to refrigerated air (RA, 0.56 °C), controlled atmosphere (CA, 0.56°C , 2% O_2, 1% CO_2), or CA with low doses of ozone gas (50 - 87 ppb) storage in a commercial facility for 30 weeks. The authors found that continuous gaseous ozone application decreased *L. innocua* population on 'Fuji' apples to 1.0 Log_{10} CFU/apple after 30 week storage, and suppressed apple native flora.

The possible mechanism of O_3 in inducing disease resistance in fruit is through the accumulation of phenolic compounds and down regulation of defence-related genes (Bambalele *et al.*, 2021). Liu *et al.*, (2021) investigated the effect of aqueous ozone treatment on the degradation of cell wall polysaccharides in fresh-cut apple during cold storage. The authors found that the aqueous ozone treatment maintained the cell wall structure and inhibited softening of the fresh-cut apple. However, Karagiannis *et al.*, (2020) found for the first time that cold storage in an atmosphere enriched with ozone (O_3) induced scald symptoms in 'Granny Smith' apple fruits during subsequent ripening at room temperature. Ozonizing the air of cold storage rooms at 0.4 ppm has also been reported to reduce ethylene production of apples without any adverse impact on fruit quality.

2.7. Electrolysed water treatment (EW) – An overview

Electrolysed water (EW) is an emerging antimicrobial agent that is used as a substitute for chlorine-based sanitizers (Y. Chen *et al.*, 2019; T. Fang *et al.*, 2013; Hricova *et al.*, 2016; Huang *et al.*, 2008; Rahman *et al.*, 2016). Electrolysed water is generated by passing electric current (~ 9–10 V electric potential) from a dilute salt solution (<0.1 g/100 mL) in an electrolysis chamber with an anode and a cathode which is divided by a diaphragm membrane as represented in Figure 2.10 (Fang *et al.*, 2016, Colangelo *et al.* 2015)). The most common salt used in this process is NaCl, but a mixture of KCl and $MgCl_2$ is also used (Colangelo *et al.*, 2015; Youssef and Hussien, 2020). In the process, two types of water possessing different characteristics are generated. An alkaline electrolysed solution (pH 11-13and oxidation-reduction potential (ORP), −795 to -900mV), is produced from the cathode side. An acidic electrolysed solution is produced from the anode side and has a high ORP (from > 1000 mV), presence of hypochlorous acid (HOCl), and strong bactericidal effect (Huang *et al.*, 2006; Huang *et al.*, 2008; Rahman, Khan, & Oh, 2016). Hypochlorous acid (HOCl) is produced during the electrolysis of saline-added water, and the amount of HOCl increases in response to the amount of added NaCl (Hussain *et al.*, 2018). However, the production of EW can be modified to reduce the

presence of HOCl and still maintains its effectiveness for microbial inactivation to reduce the health concern of chlorinated water (Kim and Yousef, 2000). Some machines usually do not have a diaphragm membrane, and neutral electrolysed water at pH: 7-8, ORP: 750 mV) is generated because HCl formed at the anode side neutralizes the NaOH at cathode side (Guentzel *et al.*, 2011). Among all these types, acidic EW has got the most attention due to its highly efficient antimicrobial activity principle. The different types of EW, their properties and efficacy will be discussed further in the following subsections. Table 2.6 represents the physical properties of different types of electrolysed water.

Table 2.6. Physical properties of different types of electrolysed water

Electrolysed water type	Abbreviation	pH	ORP(mV)	Chlorine content (mg/L)	References
Acidic electrolysed water	AEW	2-3	>1000	20-60	(Han *et al.*, 2017).
Slightly acidic electrolysed water	SAEW	5-6.7	>900	10-30	(Iram *et al.,* 2020)
Alkaline electrolysed water	AIEW	11-13	-795 to 900	N/A	(Shimamura *et al.*, 2016)
Neutral electrolysed water	NEW	7-8	750	50	(Iram *et al.*, 2021)

2.7.1. Types of electrolysed water

2.7.1.1. Acidic electrolysed water (AEW)

Acidic electrolysed water (AEW) was first developed in Japan and was approved as a food additive by Japan and the USA more than 10 years ago (Park *et al.*, 2004a; Rahman *et al.*, 2016a). It is used as a sanitizer in the food industry to reduce or eliminate bacterial populations on food products, food-processing surfaces, and non– food contact surfaces (Al-Haq *et al.*, 2005; Huang *et al.*, 2006; Hung *et al.*, 2010a). Acidic electrolysed water is produced from the anode side and contains high dissolved oxygen, free chlorine and is characterized by a low pH (2 - 3) and a high oxidation-reduction potential (ORP> 1000 mV) (Huang *et al.*, 2008). During electrolysis, the dissolved salt separates into negative (Cl^-) and positive ions (Na^+). At the same time, hydrogen ions (H^+) and hydroxyl ions (OH^-) are also formed. Thus, the negatively charged ions (Cl^- and OH^-) move to anode and give up electrons and become oxygen gas (O_2) and chlorine gas (Cl_2). Other important products formed at the anode are hypochlorous acid (HOCl), hypochlorite ions

(OCl^-), and hydrochloric acid (HCl) (Huang *et al.*, 2008).

The efficacy of AEW on reducing foodborne pathogens (*e.g. Escherichia coli*, *Listeria*, *Bacillus cereus* and *Salmonella typhimurium*) on fresh produce has been revealed in previous studies (Russell, 2003; Park *et al.*, 2004a; Ovissipour *et al.*, 2015; Li *et al.*, 2017; Mikš-Krajnik *et al.*, 2017; Chen *et al.*, 2019b; Guerra Sierra *et al.*, 2019). This effect is attributed to its low pH (2-4) and high oxidation-reduction potential (ORP), greater than 1000 mV, and because it contains active oxidants such as hypochlorous acid (Kim *et al.*, 2000). Some theories suggest the low pH, high ORP combined with active chlorine is the main reason behind the bactericidal activities (Park *et al.*, 2004b). The low pH makes the cells more susceptible to active chlorine and as a result more HOCl molecules enter through the cell membrane. Oxidation from high ORP damages cell membranes, and it has also been hypothesized that high ORP can change the normal electron flow of the cell (Hricova *et al.*, 2016). Besides the help of low pH, active chlorine compounds can destroy the structure of cell membranes. After entering cells, active chlorine compounds can react with the nucleic acids or destroy the key enzymes which are important for the normal metabolic functions (Kim *et al.*, 2000). The high ORP can also lead to the destruction of cell metabolism by reducing free radicals in microbial systems.

One interesting application of AEW was established by Han *et al.*, (2017). Biofilm formation is one of the most common problems in dealing with foodborne pathogens, as it has been established that more than 60% of foodborne microorganisms can form biofilm. Biofilm formation is problematic as a small proportion of viable cells can lead to biofilm formation and contamination of food products showed that acidic electrolyzed water with pH around 2–3 can be effective against biofilm-forming bacteria (Han *et al.*, 2017).

Table 2.7 presents a summary of studies of the effect of AEW on various microorganisms.Studies have shown that AEW is highly effective in reducing or eliminating foodborne pathogens on kitchen boards and on various food products, such as fish, poultry, fruits, and vegetables, on which it reduced populations of pathogens to undetectable levels (Hricova et al., 2016). In recent years, acidic electrolyzed water is investigated as one of the advanced technologies for non-thermal food processing.

Table 2.7. Antimicrobial effect of AEW on various microorganisms

Micro-organism	Reference
Bacteria	
Bacillus cereus	Zhang *et al.*, (2016); Li *et al.*, (2017); Hussain *et al.*, (2018)
Listeria monocytogenes	Ovissipour *et al.*, (2015, 2018); Han *et al.*, (2017); Tango *et al.*, (2017); Chen *et al.*, (2019a)
Escherichia coli 0157:H7	Venkitanarayanan *et al.*, (1999); Park *et al.*, (2004); Ogunniyi *et al.*, (2021); Al-Qadiri *et al.*, (2016)
Staphylococcus aureus	Russell, (2003); Issa-Zacharia *et al.*, (2010)
Fungi	
Botrytis	Guentzel *et al.*, (2011); TU *et al.*, (2016); Guerra Sierra *et al.*, (2019); Guentzel *et al.*, (2010a, 2011b)
Penicillium	(Okull and LaBorde, 2004b)

2.7.1.2. Alkaline electrolysed water (AIEW)

Alkaline electrolysed water (AIEW) has a strong reducing potential that may lead to a reduction of free radicals in biological systems. In this regard, it is used for cleaning the most common surfaces and things that do not require harsh antimicrobial treatments. It may also be useful in the treatment of organ malfunctions (Kim & Yousef, 2000). AIEW is formed at the cathode of the electrolysis chamber where positive ions such as H^+ and Na^+ are moved toward the cathode where they take electrons and become hydrogen gas (H_2) or sodium hydroxide (NaOH) (Huang *et al.*, 2008). Alkaline electrolysed water (AIEW) has a high pH ranging from 11.0-13, low ORP ranging from -795 to -900 mV, lower dissolved oxygen, and higher hydrogen than tap water or deionized water (Shimamura *et al.*, 2016). The pH also plays a role in the disinfection as it is greater than 11 in the case of alkaline electrolysed water. The presence of dilute NaOH is linked with a high surface decontamination effect. While alkaline electrolysed water can be effective for bactericidal effect, it is not as effective as acidic electrolysed water and therefore has fewer applications for the industry and research. Yang *et al.*, (2020) investigated the removal of typical pesticide residues in kumquat by alkaline electrolysed water solutions with pH = 11.0 and pH = 12.5. The authors found that compared with other washing solutions, the pH 12.5 electrolysed water was the most effective media for pesticide residue removal. Moreover, a washing duration of 20 min was optimum. The authors thus concluded that alkaline electrolysed water in higher pH provides a potential removal strategy for pesticide residues on fruit surfaces.

2.7.1.3. Neutral electrolysed water (NEW)

Neutral electrolysed water (NEW) is formed by mixing the acidic and alkaline EWs or by using an electrolysis chamber without a diaphragm, and the resulting positive and negative ions react with each other to form a nearly neutral solution (7–8 pH) with around 700 mV ORP (Iram *et al.*, 2021). Another way to produce NEW is by mixing the electrolysed water with OH^- ions. NEW is considered the least effective in antimicrobial activities amongst all the three different types of electrolysed water. However, it is less corrosive and can enter the microbial cell without much hindrance. Therefore, it has also been explored extensively where corrosiveness is a concern (Guentzel *et al.*, 2011). Among various concerns related to EW, one of the most prominent is storage which decreases the effectiveness of AEW with respect to time. NEW, on the other hand, has a longer shelf life as compared with EW. Therefore, NEW is advantageous over EW where long storage of water is required.

In a study by Han *et al.* (2018), meat products such as Atlantic salmon and pork products were evaluated by treating them with NEW. In the case of Atlantic salmon, NEW

with 6.8 pH showed a log reduction of 5.6 CFU/ ml for *L. monocytogenes*. The pork products, however, only showed 0.8 CFU/ml log reduction for different foodborne bacterial species (Han *et al.*, 2018). This shows the variable effect of NEW for different microbial species. Nevertheless, it can be seen that NEW has higher antimicrobial properties as compared with strong acidic and alkaline electrolyzed water.

All three types of EW are currently being explored for their effectiveness in the biological systems as antimicrobial agents. Different applications of electrolyzed water include major types of pathogenic agents including bacteria, fungi, viruses, protozoa, algae, and nematodes. The advantages and disadvantages of EW treatments are further listed on Table 2.8.

Table 2.8. Advantages and disadvantages of electrolysed water treatment.

	Advantages	Disadvantages
Electrolyes water treatments (EW)	1. Electrolyzed water treatment is not harmful to human health as compared to chlorine (Colangelo *et al.*, 2015; Hricova *et al.*, 2016). 2. It can be produced on-site (Colangelo *et al.*, 2015). 3. When EW comes in contact with organic matter or is diluted with tap water, it is converted back to ordinary water (Hricova *et al.*, 2016; Rahman *et al.*, 2016b). 4. In addition, EW is convenient to prepare and the cost of applying EW is inexpensive (Colangelo *et al.*, 2015). 5. It impacts the environment less adversely because it is environment friendly as it is generated by the electrolysis of only water and a dilute salt solution (Issa-Zacharia *et al.*, 2010). 6. As a non-thermal method, the use of EW does not result in changes in nutritional and sensory attributes of food products, which are brought about by heat-treatment (Lado and Yousef, 2002).	1. The main disadvantage of electrolyzed water is the rapid loss of its antimicrobial activity in a short time because of chlorine loss (Rahman *et al.*, 2016b; Shimamura *et al.*, 2016; Guerra Sierra *et al.*, 2019). 2. Its effectiveness can be reduced by the presence of protein. This is mainly due to protein reacting with chlorine (Rahman *et al.*, 2016a). 3. With time, the bactericidal activity of AEW is reduced due to chlorine loss (Youssef and Hussien, 2020). 4. Acidic electrolysed water (AEW) contains free chlorine, which can be phytotoxic to plants and damage plant tissue (Rahman *et al.*, 2016b)

2.7.2. Factors affecting activity of electrolysed water (EW)

Literature has reported that several factors influence the EW properties that determine bactericidal efficacy. In Figure 2.8, available chlorine concentration (ACC), pH, and oxidation-reduction potential (ORP) are considered the main factors directly affecting EW's sanitizing effectiveness.

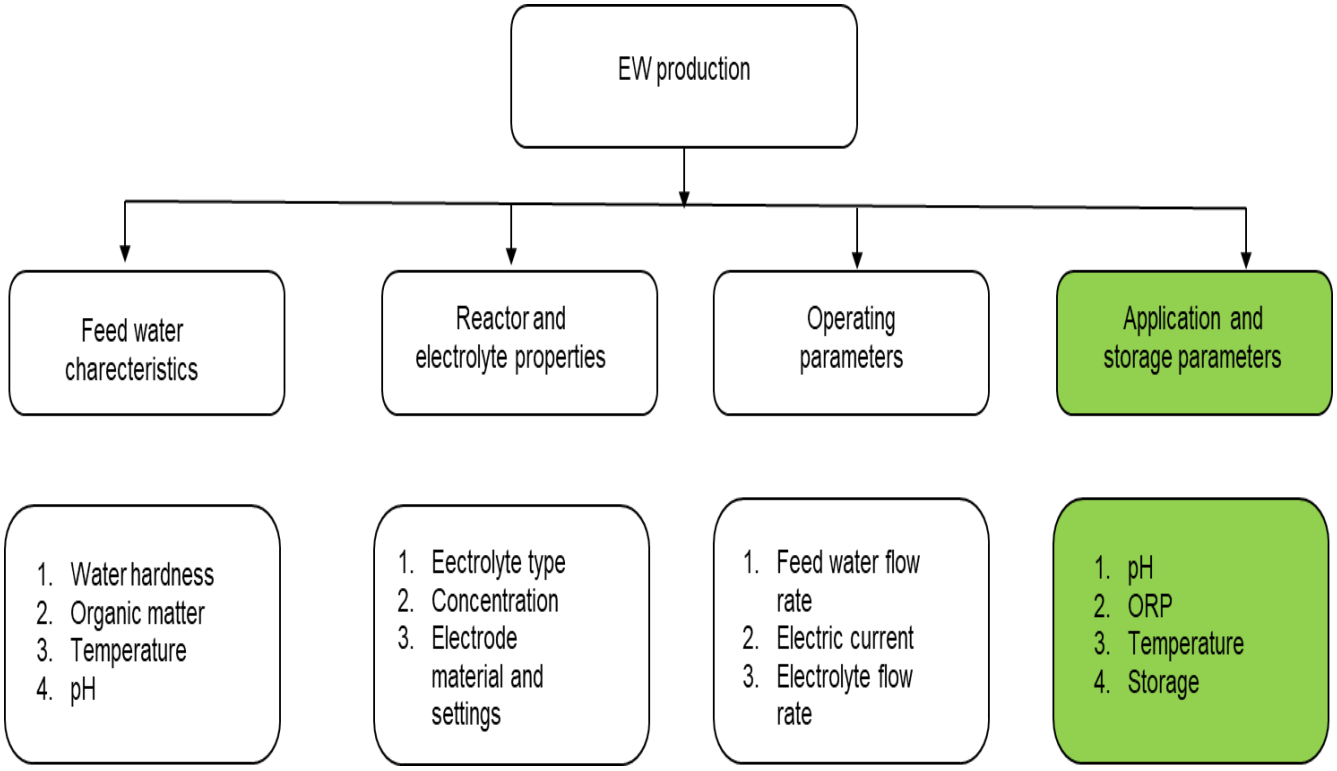

Figure 2.8. Factors affecting the efficiency in the production and application of EW (Adapted from Ampiaw *et al.,* 2021)

Available chlorine concentration (ACC)

The main factor contributing to the disinfecting potential of electrolyzed water is deduced to be the oxidizing power of available chlorine. According to Koseki and Itoh, (2000), there is no difference in the disinfectant effects between EW with high ACC (40 ppm) and low ACC (0.4 ppm). A similar effect was detected in EW with 0 ppm of ACC, a solution that seemed to be the same as hydrochloric acid. Moreover, tap water with pH adjusted to 2.4 showed the same disinfectant effect as that of EW (Koseki and Itoh, 2000). The effect of available chlorine concentration is also assessed in multiple research reports and more than 20 ppm is correlated with effective inactivation of microorganisms (Quan *et al.*, 2010).

Oxidation-Reduction potential

Oxidation-reduction potential (ORP) plays a major role in determining the efficacy of electrolysed water treatments. The ORP value decreases markedly when the pH is increased. A high ORP results in oxidation affecting the outer layer of microorganisms, causing further oxidation reactions which result in the ultimate destruction of the metabolic pathway in the microorganism cell, leading to its inactivation (Liao *et al.*, 2004). It has also been hypothesized that high ORP can change the normal electron flow of the cell (Hricova *et al.*, 2016). Koseki et al. (2004) stated that ORP is not the principle factor responsible for the sanitization process. The authors observed that ozonized water with a high ORP showed lower germicidal efficacy when compared to acidic electrolysed water which has a low ORP and attributed the strong antimicrobial effect of AEW to the ACC, mostly HOCl.

pH

The pH of EW is an important parameter since it plays a significant role in in the determination of ORP and ACC values. When the pH is high, ACC occurs in the form ClO^- and the ORP is low. Therefore ACC values are presumed high, however, the ORP will decrease because of the high pH. All these parameters, i.e. pH, ORP and ACC (as HOCl or ClO^-) have a powerful influence on the germicidal efficiency of electrolysed water (Ampiaw *et al.,* 2021).

Alkaline electrolysed water is characterised by an alkaline pH and negative ORP values, with hypochlorite (OCl^-) and small amounts of sodium hydroxide (NaOH) in solution, which improve the removal of the organic matter. In acidic electrolysed water, the pH values range from 2-3, and chlorine is predominantly in the form of hypochlorous acid (HOCl), which presents excellent sanitizing activity. The pH value alters the formation of chlorine species. However, low pH can cause corrosion of equipment and utensils, and also to the health risks of the use of chlorine gas (Colangelo *et al.*, 2015; Hricova *et al.*, 2016; Rahman *et al.*, 2016b; Shimamura *et al.*, 2016; Guerra Sierra *et al.*, 2019).

Rahman *et al.* (2010) found that the antimicrobial efficacy of EW was significantly diminished at a pH of 9.0 because the ORP and ACC values decreased significantly with an increased pH from the acidic region (2.5) to the basic region (9.0). In another, Park *et al.* (2004) investigated the effect of pH and ACC of acedic electrolysed water on the inactivation of *Listeria monocytogenes* and *Escherichia coli.* The authors observed that within a pH range of 3-7, the AEW was extremely efficient in retarding pathogenic activity when adequate amounts of ACC was present. Other studies have attributed the EWs

ability to reduce the bacterial population to pH, which increases the susceptibility of the external surface of the pathogens to HOCl.

Storage conditions

Storage conditions affect the chemical and physical properties of electrolysed water treatments (EWs). EWs stored under open conditions have the highest chlorine loss which affects the efficacy of the treatments. Chlorine loss is primarily through the evaporation of dissolved chlorine gas and ensuing HOCl decomposition. Chlorine loss causes loss of bactericidal activity within time. According to Guentzel *et al.*, (2011), acidic electrolyzed water returns to its original state, as there seems to be rapid chlorine loss due to the evaporation of chlorine gas and HOCI decomposition. Under closed conditions, the EWs are much more stable and chlorine self-decomposition could be the mechanism of chlorine loss.

White, (1998) reported complete chlorine loss in acidic electrolysed water kept in an open environment after 30 h of shaking and 100 h when agitated. Rahman *et al.* (2012) investigated the effect of storage on slightly acidic electrolysed water under closed and open conditions at a pH of 6.8-7.4. The authors observed a gradual decrease from the initial ACC of 10 mg/Lto an ACC of 0 mg/Lwithin 7 days in an open dark environment and a decline from an ACC of 10 mg/L to an ACC of 0 mg/L in 21 days under closed dark conditions. It has also been reported that lower temperatures are conducive to EW storage. Fabrizio and Cutter, (2003) showed that the physicochemical properties of EW stored at 4 °C exhibited more stability that EW stored at 25 °C and exhibited effective bactericidal activity for over 12 months. However, it has been proven that EW exhibits different properties under diverse conditions. For instance, Nagamatsu *et al.* (2002) and Cui *et al.* (2009) reported that neutral electrolysed water (NEW) had higher stability than acidic electrolysed water (AEW) throughout the storage period. Cui *et al.* (2009) reported no change in the Ph and electrical conductivity of NEW and AEW after 30 days of storage, however, when AEW was kept under open conditions there was a 22% decrease in ORP and 100 % in ACC was observed.

2.7.3. Decontamination effect of electrolysed water treatment on apple fruit during post-harvest handling

The post-harvest application of EW has been investigated in controlling microbial contaminations in apple fruit (Table 2.8). For instance, Okull and LaBorde, (2004b) investigated the activity of electrolyzed water (EW) against *P. expansum* in suspension on wounded apples. Full-strength and 50% EW water decreased viable spore populations by greater than 4 and 2 log units, respectively. Although EW water did not prevent lesion formation on fruit previously inoculated with *P. expansum*, cross-contamination of wounded apples from decayed fruit or by direct addition of spores to a simulated dump tank was substantially reduced. Electrolyzed water, was therefore proven, to have the potential as an alternative to chlorine disinfectants for controlling infection of apples by *P. expansum* during handling and processing operations. Similarly, Nimitkeatkai and Kim (2009) observed the effect of EW on the washing of apples. A strong acidic EW with pH 2.8 and a weak acidic EW with pH 6.5 was used. The authors assessed the efficacy of both solutions and the better choice was to wash the apple with weak acid EW (either 2 or 5 min) or strong acidic EW for 2 min to preserve the sensory quality of the apple . However, there is no report on the effect of EW on quality attributes of apple fruit such as colour, antioxidant activity and bioactive compounds.

Furthermore, 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 in the concentration of some pesticide residues on the fruit. In another study by Graça *et al.* (2010) neutral electrolyzed water (NEW) and acidic electrolyzed water (AEW) were used to inactivate food-borne pathogens on the surface of fresh-cut apples, pears, and oranges. Based on the outcome of this study both NEW and AEW were able to reduce the microbial populations, and treatment with AEW specifically had a higher efficacy. Furthermore, AEW at 50 and 100 mg/L of free chlorine were used to treat apple slices inoculated with *E. coli, Listeria*, or *Salmonella* and significantly decreased the populations of the pathogen, when compared to samples treated with sodium hypochlorite solution and distilled water (Graça *et al.*, 2011).

Moreover, Kim and Hung (2014) studied the effect of EW on enzymatic browning in cv. "Red Delicious" apples. Polyphenol oxidase (PPO) activity, hue angle, chroma and browning index (BI) of the alkaline electrolyzed (EW) water-treated apples were compared with apples treated with other anti-browning agents (ascorbic acid, citric acid and sodium metabisulfite). Alkaline EW was shown to reduce PPO activity by about 66%. Apples treated in alkaline EW water for 5 min had less reduction on hue angle (97.0 - 91.1) after 24 h of storage than apples treated with deionized water (93.4-83.7) and hence less

brown. This study demonstrated that alkaline EW water can be a promising treatment solution to prevent browning and thereby enhance the quality of fresh-cut apples. Other studies have shown that EW is highly effective in reducing or eliminating foodborne pathogens in citrus (Fallanaj *et al.*, 2016), strawberries (Hung *et al.*, 2010a), pear, (Al-Haq *et al.*, 2002) fresh-cut vegetables (Hao *et al.*, 2011) on which it reduced populations of pathogens to undetectable levels.

Table 2.9. Overview of studies studies researching on microbial inactivation and quality of apple fruits by electrolysed water treatments (EW)

Apple Cultivar	Type of EW	Initial microflora	Treatment conditions	Microbial observation	Quality observation	References
Fuji apples	Strong acidic (pH 2.8) EW and weak acidic (pH 6.5) EW, each containing 50 μL・L^{-1} free chlorine	N/A	Treatment concentration: 50 Treatment time: 2 and 5 min Storage Temperature: 10 °C	• Washing in strong acidic EW for 5 min was effective in reducing microbial population.	• Apples washed in the EW for 5 min had less hue angle value throughout storage period and lower sensory evaluation score at the end of storage.	(Nimitkeatkai and Kim, 2009)

Table 2.9. Continued

Apple Cultivar	Type of EW	Initial microflora	Treatment conditions	Microbial observation	Quality observation	References
Fresh-cut apples	Neutral electrolyzed water (NEW) and acidic electrolyzed water (AEW)	*Listeria innocua* and *Escherichia coli O157:H7*	Treatment concentration: 100 and 200 ppm	• The bacterial population was significantly reduced in all fruits treated with AEW at 200 ppm of free chlorine. • Treatments with the AEW with 100 ppm free chlorine resulted in reductions higher or similar to NEW	N/A	(Graça *et al.*, 2010)
Fresh cut 'Royal Gala' apple slices	Acidic electrolysed water (AEW- pH 2.87 ± 0.08, ORP 1146 ± 7 mV) and Neutral electrolysed water (NEW- pH 8.43 ± 0.45, ORP 771 ± 11 mV)	*E. coli O157:H7 NCTC 12900, L. innocua CECT-910 and S. choleraesuis*(10^7 CFU/mL)	Treatment concentration: 50 and 100 mg/L Treatment time: 5 min at 150 rpm agitation	• The highest population reduction of *E. coli* 30 min after treatment was observed in apple slices washed with AEW100 and AEW50 with values of 2.47 and 2.09 log CFU/g respectively. • *L. innocua* was reduced more than 1.20 log CFU/g with AEW100.	N/A	(Graça *et al.*, 2011)

Table 2.9. Continued

Apple Cultivar	Type of EW	Initial microflora	Treatment conditions	Microbial observation	Quality observation	References
Red Delicious apples	Neutral electrolysed water (NEW- pH of 7.88, an ORP of +801 mV and a free chlorine concentration of 472 mg/L)	*A. acidoterrestris* (ATCC49025) (10^7 CFU/mL)	Treatment concentration: 50 and 200 mg/L of free chlorine (Designated as NEW50 and NEW200) Treatment time: 1, 3 and 5 min	• Viable spore counts in test suspensions were significantly reduced after exposure to NEW (200 mg/L. However, NEW (50 mg/L free chlorine) and was unable to significantly reduce the number of viable spores during the same exposure period. • More than 5 log reduction in spore counts was achieved by NEW solution containing 200 mg/L free chlorine after 5 min of exposure. • Exposure to NEW solutions for 3 min yielded more than 4 log reductions in the number of viable spores on apple surfaces.	N/A	(Torlak, 2014)

Table 2.9. Continued

Apple Cultivar	Type of EW	Initial microflora	Treatment conditions	Microbial observation	Quality observation	References
Fresh cut 'Royal Gala' apples	Acidic electrolyzed water (AEW-pH of 2.87 (±0.05), an ORP of 1113 ±2 mV and a free chlorine content of 102 ±3 mg/L and Neutral electrolyzed water (NEW-pH of 7.95 ± 0.06, an ORP of 757 ± 4 mV and a free chlorine content of 101 ± 2 mg/L.)	*Candida sake, Hanseniaspora uvarum, Pichia fermentans* and *Metschnikowia pulcherrima* (10^7 CFU/Ml)	Treatment time: agitated for 5 min at 150 rpm in an orbital shaker	• The estimated parameters values of initial microbial reduction caused by the AEW and NEW decontamination methods were, 1.86 and 1.96 Log CFU/g, and the half-life time constant (e) values were 4.38 and 4.22, respectively.	N/A	(Graça *et al.*, 2020)

2.8. Conclusion

Antioxidant activity and polyphenols are abundant in apple fruits. There are many biological activities within apples that result in increased levels of phenolic compounds and antioxidants, which may help to reduce the likelihood of developing cancer, hypertension, diabetes, and heart disease. Overall, apple fruit contains many beneficial compounds that are essential for human health. With an average export share of 66% in South Africa, it is imperative that apple producers have alternative post-harvest treatment strategies for the control of pathological and physiological disorders to safeguard the apple fruit industry by ensuring a continuous supply of quality fruit to its export markets and to provide consumers access to fresh fruit throughout the year. Electrolysed water is an emerging antimicrobial treatment method that has recently gained interest due to its confirmed applications in the food industry. Further, it has been shown that EW is more effective in suspensions than on food and equipment surfaces. Even though it is not as effective with suspensions as with surfaces, EW is an effective alternative to thermal processing when it comes to the disinfection of fruit. Recent research reports show that electrolysed water is a powerful antimicrobial agent that can be used in combination with physical and chemical treatments to increase the effectiveness of antimicrobial effects without negatively impacting fruit quality.

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CHAPTER 3

Curative efficacy of slightly alkaline and acidic electrolyzed water treatment against *B. cinerea* and *P. expansum* on 'Granny smith' apples

Abstract

Botrytis cinerea and *Penicillium expansum* are one of the major causes of rotting in apples during postharvest storage and cause a significant economic loss. Over the years, intensive fungicide applications have been reported but the need for alternative techniques arises due to toxicological problems of repeated fungicides in humans and the environment. This study aimed to investigate alternative approaches such as electrolyzed water (EW) treatments for decay management of 'Granny Smith' apples. Two different sets of experiments were set up for this study. In experiment 1, the curative efficacy of slightly alkaline electrolyzed water (SAI-EW) (50, 100, 200, 300, 400 and 500 mg L^{-1}) treatment against *B. cinerea* on 'Granny Smith' apple was investigated. In experiment 2, the curative efficacy of acidic electrolyzed water (AEW) treatment (100, 200 and 300 mg L^{-1}) and slightly alkaline electrolyzed water (SAI-EW) treatment (100, 200 and 300 mg L^{-1}) against *B. cinerea* and *P. expansum* on 'Granny Smith' apples was investigated. 'Granny Smith' apple were inoculated on opposite ends with a spore concentration of 1×10^4 spores L^{-1} of *B. cinerea* and 1×10^5 spores L^{-1} of *P. expansum* using a 3 mm cork-borer. The inoculants were allowed to air dry for 5-6 h and incubated at 20 °C for 20 h to allow for spore germination. In experiment 1, measurements of the lesion zones were carried out weekly for 5 °C, and after two days of storage at 24 °C for shelf life study and in experiment 2, every 3 days for apples stored at 15 °C**.** The SAI-EW treatments against *B. cinerea* resulted in significant ($p \leq 0.05$) reduction in lesion zones of decay across all concentrations in comparison to the control. The curative efficacy of the treatments was most effective at the highest concentration of 500 mg L^{-1}, followed by 400 mg L^{-1}, and 300 mg L^{-1} for treated apples. The AEW treatments against *P. expansum* and *B. cinerea* resulted in a significant ($p \leq 0.05$) reduction in lesion zones compared to the control. This result indicated that both SAI-EW and AEW were effective as an antifungal treatment against *B. cinerea* and *P. expansum.* These findings therefore suggest the potential of combining lower concentrations of SAI-EW and AEW with other hurdle techniques for a synergistic antifungal effect and better preservation of good apple quality attributes.

Keywords: Antifungal activity, fruit quality and safety, lesion area, synergistic effects

3.1. Introduction

Apple fruits are usually stored for long durations at low temperatures after harvest. During cold storage losses of economic importance are produced by several decays due to fungal disorders. *Penicillium expansum* and *Botrytis cinerea* are well-known post-harvest pathogens (Morales *et al.*, 2010; Bui *et al.*, 2019). *B. cinerea,* which is the main cause of development grey mould disease, is the second most important phytopathogenic fungus around the world (Dean *et al.*, 2012) and can infect many fruits at harvest or earlier and during the post-harvest stage. The infection primarily enters apple fruit through wounds and other surface defects that occur during and after harvest and in storage, creating infection that manifests in decay (Zhao *et al.*, 2010). *P. expansum* can infect a wide range of fruit to cause blue mould, and produces the mycotoxin patulin (He *et al.*, 2019), which can induce immunological, neurological, and gastrointestinal diseases leading to serious health problems in humans. In addition, *P. expansum* infects apple fruit primarily through wounds caused by stem punctures or bruises occurring at harvest or during post-harvest handling (Vico *et al.*, 2014; Yu *et al.*, 2020). Both these pathogens account for more than 25% of total production losses in developed countries and more than 50% in developing countries (Nunes, 2012).

'Granny Smith' is the most important apple cultivar grown in South Africa. One of the main problems that occurs during its post-harvest storage is the damage due to *B. cinerea* and *P. expansum* and may result in up to 50 % losses during storage (Mbili *et al.*, 2017). To date, the use of synthetic chemicals such as fungicides is a primary method of control of post-harvest fungal decay of apple fruit (Dean *et al.*, 2012). However, several fungicides are not used for post-harvest treatment or have been removed from the market due to possible toxicological risks to humans and the environment. Alternative methods of control are needed because of the negative public perceptions about the use of fungicides, development of resistance to fungicides among fungal pathogens, and high development costs of new chemicals. In recent years, the use of electrolyzed water treatments to control post-harvest diseases of fruits has become an important field for research (Venkitanarayanan *et al.*, 1999; Hung *et al.*, 2010; Ferri *et al.*, 2016).

Electrolyzed water (EW) treatment is an emerging hurdle technique, which is cost effective and environmentally friendly (Huang *et al.*, 2006). It has been regarded as a new sanitizer and cleaner in the food industry. This is due to its simplicity of production as well as application. Over the last years, research studies have been conducted on the antimicrobial activity of EW on different food products. Post-harvest application of EW has been shown to successfully control microbial contaminations in various circumstances on citrus, strawberries, pears, and fresh-cut vegetables (Ferri *et al.*, 2016; Qi *et al.*, 2018; Guerra Sierra *et al.*, 2019).

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 in the concentration of some pesticide residues on the fruit. Guerra Sierra et al. (2019) reported on the antifungal activity of electrolyzed water against strawberry postharvest moulds and concluded that the EW treatment can be employed as a useful disinfectant for strawberry moulds, and that it holds potential for the development of environmentally friendly products for the control of fungi in fruits in the storage phase. 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.

There is currently no report related to the application of EW to control the growth of *B. cinerea* and *P. expansum* in 'Granny Smith' apple fruit in South Africa. Despite the high demand for 'Granny Smith' apple due to its antioxidant properties, and its susceptibility to quality loss during post-harvest handling and storage, there has not been much done to apply physical pre-treatments using EW. Therefore, the current study was carried out in order to investigate the curative efficacy of slightly alkaline electrolyzed water (SAI-EW) treatment and acidic electrolyzed water (AEW) treatments against *B. cinerea* and *P. expansum* on 'Granny Smith' apples stored at 5 °C for 21 days plus two days of accelerated storage at 24 °C, and 15 °C for 9 days, respectively.

3.2. Materials and Methods

3.2.1. Plant material

In this study, two consecutive experiments were conducted. Fresh apple (cv. Granny Smith) were obtained at commercial maturity from a commercial pack house, 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 for a week prior to treatment.

3.2.2. Pathogen Isolation

The fungal pathogen *B. cinerea* (Accession number: PPRI 7338) was obtained from the Agricultural Research Council- Plant Protection Institute, Pretoria. South Africa. The strains were isolated from infected plums. *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 agitated the plates to dislodge the spores. The final inoculum concentration was adjusted to 1×10^4 conidia mL^{-1} using a haemocytometer.

The fungal pathogen *P. expansum* (Accession number: PPRI 5944) was obtained from the Agricultural Research Council- Plant and Protection Institute, Pretoria. The strains were isolated from infected apples, tested for pathogenicity. Stock cultures were maintained on PDA media by sub-culturing every 2 week. The cultures of *P. expansum* 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 1×10^5 conidia mL^{-1} using a haemocytometer.

3. 2.3.Electrolyzed water preparation

In this study, the electrolyzed water was generated using the ELA-12 000ANW system (ECA Technologies, Envirolyte, South Africa). The electrolysed water was generated by electrolysis of hydrochloric acid in the range (0.05 %), sodium chloride (0.26 %) and water (99.69 %). The electrolyte flow passed through an electrolytic cell at a rate of 2 mL min^{-1}, at a setting current of 3.8–3.9 V and amperage 10 A. The pH and ORP were measured immediately after preparation and right before experiments to confirm that they were not significantly changed using pH meter (D-22, Horiba, Kyoto, Japan) and ORP meter (HM-60V, TOA Electronics Ltd., Tokyo, Japan), respectively. The AEW obtained from the anode consisted of: available chlorine concentration (ACC) of 500 mg L^{-1}, ORP > 1000 mV, and pH = 3. The SAI-EW obtained from cathode anode consisted of: available chlorine concentration (ACC) of 500 mg L^{-1}, ORP > -800 mV, and pH = 6.7. Both AEW and SAI-EW were collected at low temperature (4 °C) and used immediately in this study. Food grade sodium hypochlorite (NaOCl, 11.5% M/V) solution was purchased from Protea Chemicals, Sandton, South Africa**.** All treatment solutions were diluted to desired concentrations prior to dipping the apples and non-treated samples were considered as control. The dipping duration selected was based on the average pack-house processing time for apples. Description of treatments and their abbreviations as used further in this study are presented in Table 3.1.

Table 3.1. Electrolyzed water concentration used to treat 'Granny Smith' apple fruit.

Treatment(s)		
Active Compound	**Concentration (ppm)**	**Treatment(s) Abbreviation**
SAI-EW	50	EW1
SAI-EW	100	EW2
SAI-EW	200	EW3
SAI-EW	300	EW4
SAI-EW	400	EW5
SAI-EW	500	EW6
AEW	300	EW1a
AEW	200	EW2a
AEW	100	EW3a
Non-treated	-	Control

SAI-EW (slightly acid electrolyzed water), AEW (acidic electrolysed water)

3.2.2. Fruit treatment and storage (Experiment 1)

Samples were randomly divided into homogenous groups of twenty-one batches (box with 150 apples) representing the seven treatments and three levels (Table 3.1). 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.* 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 replicas for each batch per treatment was taken on the sampling day (1, 7, 14, and 21) for image analysis.

3.2.3. Image analysis (Experiment 1)

Visual quality assessment was done to determine the effect of electrolyzed water treatment on the lenticel 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, a digital image (2048 × 1536 pixels) of 'Granny Smith' apple was taken using a 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 μm^2) for the lesion zone was 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 resulting lesion zone was expressed in mm.

3.2.4. Fruit treatment and storage (Experiment 2)

A cork borer was used to uniformly wound the fruit (3 mm × 3 mm). Apple 'Granny Smith' were then inoculated on opposite ends with a spore concentration of 1×10^4 L^{-1} spores of *B. cinerea* and 1×10^5 L^{-1} spores of *P. expansum* and allowed to air dry for 5-6 hrs. 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. Each fruit batch was exposed to one of the treatments for a duration of time (10 and 15 min). Measurements of the inoculated/lesion zones were carried out every 3 days for a week using a measuring scale. After treatments, all the samples were stored at 15 °C and 90 ± 2% RH for 9 d and analyses were conducted in triplicate on days 0, 3, 6, and 9.

3.2.5. Statistical analysis

Data obtained was analysed by one-way ANOVA at 95 % confidence interval using Statistica Software (version 13, StatSoft Inc. TIBCO Software Inc., USA). The difference between mean values was tested using the Duncan multi-range test.

3.3. Results and Discussion

3.3.1. Experiment 1

3.3.1.1. Visual quality

The visual quality analysis was carried out to 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 concentrations affected the lenticel colour appearance during storage in comparison to the control samples. Smaller lesion areas were observed when the EW concentrations increased above 200 mg L^{-1} and curative efficacy was most effective at the concentration of 500 mg L^{-1}, followed by 400 mg L^{-1}, and 300 mg L^{-1} for treated apples (Figure 3.1 & 3.3). 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. Generally, the expansion of lesion area was accelerated under ambient storage conditions at 24°C compared to 5 °C at lower EW concentration. These results clearly showed that EW treatment applied at higher concentrations can be used to inhibit the growth of *B. cinerea* even at shelf life storage (24 °C). Ferri *et al.* (2016) evaluated two different strategies for the storage of 'Cripps Pink' apples. In the first one, the apples were sprayed with EW at 400 mg L^{-1} of free available chlorine in the field before harvest, and were then stored at 1 °C under controlled atmosphere for 4 months. In the second storage strategy, apples were not treated with EW in the field, but moved directly to the storage facility after harvest, where they were stored for 2 months at 1°C in controlled atmosphere, and then either washed with working line water, or washed by dipping in EW at 50 mg L^{-1} or washed by spraying with EW at 400 mg L^{-1}. After washing, the apples were stored at 25°C and checked every seven days. The authors showed that the percentage of rotted apples was significantly lower if the apples were treated with EW at 400 mg L^{-1} before harvest and storage temperature was found to influence decay.

On the other hand, this study demonstrated that the EW treatment (300 mg L^{-1}) was sufficient to control appearance throughout the storage time at different treatment times. 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 post-harvest storage. Similarly, Hayta and Aday, (2015) reported that EW treatment at concentrations 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 findings 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.

3.3.1.2. Efficacy of electrolyzed water (SAI-EW) treatment against *B. cinerea*

The results obtained showed that electrolyzed water treatment (SAI-EW) concentration, dipping duration and change in storage temperature had a significant effect on the lesion area of *B. cinerea (*Figure 3.1 A, B &C). After 21 days of storage at 5 °C, the lesion area doubled (50%) from the initial growth area for EW-1 and EW-2 (Figure 3.1 A). 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. A 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 the concentration of 500 mg L^{-1}, followed by 400 mg L^{-1}, and 300 mg L^{-1} for treated apples.

Furthermore, varying dipping duration at low concentrations (EW-1 and EW-2) did not make a significant difference in lesion area. The highest lesion area with *B. cinerea growth* was observed for 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 effective as a curative strategy against *B. cinerea.* However, comparing the results of 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 samples 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 EW (50-100 mg L^{-1}) to inactivate *B. cinerea* for peaches and grapes resulted in 10^6 reduction and 100% inactivation in addition to the fact that EW did not leave any chlorine residue on the fruit surface compared to using water. For the highest concentrations of EW treatments such as EW-5 and EW-6, no increase in the growth area was observed for samples under cold storage. At the end of the storage duration, the growth areas for the lower concentrations (50 mg L^{-1} and 100 mg L^{-1}) were 87 % and 78 %, respectively. The mean initial lesion area on the EW treated apple at 24 °C was 49.9 mm^2 for 15 min, whereas the initial lesion area for control fruit was 69.4 mm^2 (Figure 3.1C1).

Change in storage temperature was observed to accelerate the growth of *B. cinerea*. 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 was 69.35 mm (Figure 3.3 A, B & C). From the treated fruits, 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 increased >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 3.4). However, for similar storage duration, the lesion area for the control fruit increased more than 95%. These results clearly showed that the EW treatment could be used to inhibit the growth of *B. cinerea* even at shelf life storage (24 °C). In another study done by Jane *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 decreased 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 a 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 the inactivation of *L. monocytogenes* in fresh 'Granny Smith' and 'Fuji' apples 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. A longer contact time might increase microbial resistance, binding strength of bacteria to the product surface or might induce bacterial desiccation stress response. This could explain the beneficial effect of 10 min treatment time in comparison to 5 and 15 min treatment time. Studies are evident that EW does not have a negative impact on human health since EW can be converted back to ordinary water when there is contact with organic matter or when diluted with tap water (Hayta and Aday, 2015).

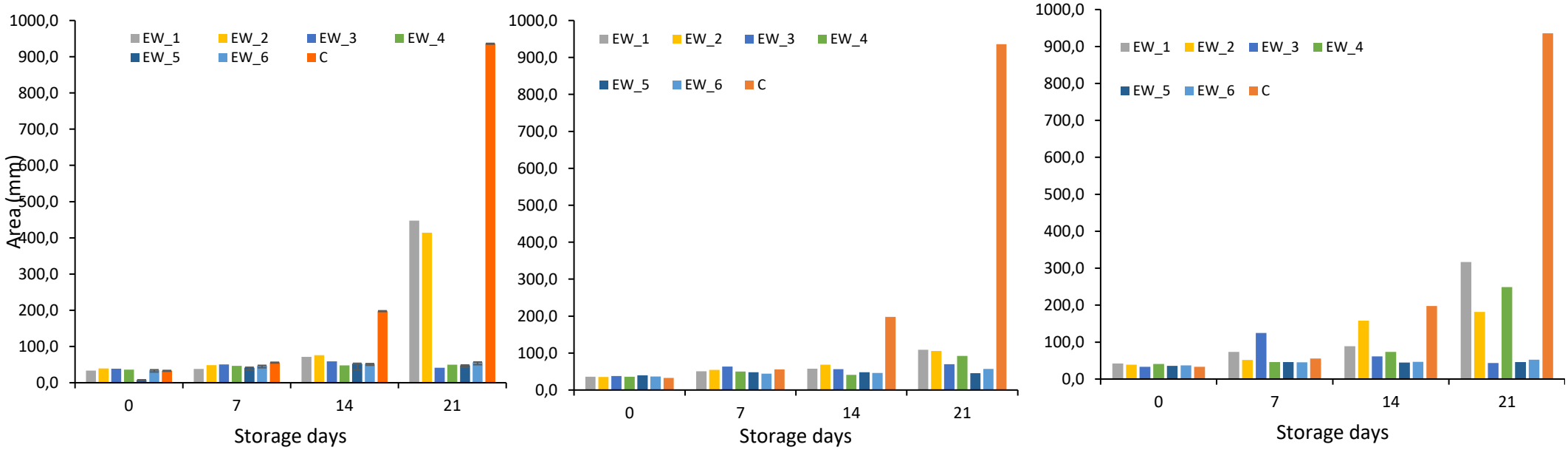

Figure 3.1. Effect of SAI-EW concentrations (Table 3.1), treatment time (5 min (A), 10 min (B) and 15 min (C)) at 5 °C and (5 min (A1), 10 min (B1) and 15 min (C1)) at 24 °C and storage duration on the growth area of *B. cinerea* on 'Granny Smith' apple stored.

Figure 3.2. Differences in lesion area/ growth of *B. cinerea* on 'Granny Smith' apple at first week (first row) and after four weeks due to the effect of different electrolyte water treatment. Second raw (5 min treatment), third row (10 min) and fourth raw (15 min) treatment after four weeks of storage at 5 °C.

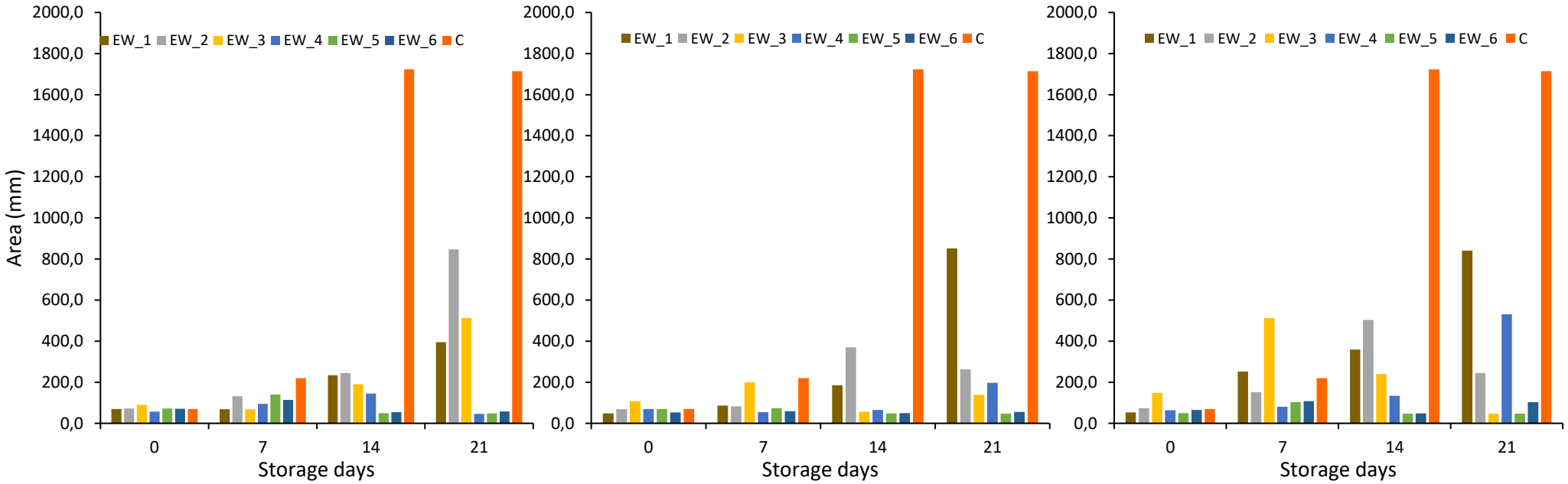

Figure 3.3. Effects of SAI-EW concentrations (Table 3.1), treatment time (5 min (A), 10 min (B) and 15 min (C)) and storage duration on the growth area of *B. cinerea* on 'Granny Smith' apples stored at 24 °C.

Figure 3.4. Differences in lesion area/ growth of *B. cinerea* on 'Granny Smith' apple at first week (first row) and after four weeks due to the effect of different electrolysed water treatment. Second row (5 min treatment), third row (10 min) and fourth row (15 min) treatment after four weeks storage at 24 °C.

These studies thus prove that electrolyzed water has potential as a disinfectant for controlling infection of apples. In the present study, slightly alkaline electrolyzed water-NaCl treatment was effective as an anti-fungal treatment against *B. cinerea*. These findings suggest the potential of combining lower concentrations of SAI-EW with other hurdle techniques for a synergistic antifungal effect and better preservation of good fruit quality attributes. A major advantage of using electrolyzed water during postharvest handling is the lower adverse impact it has on the environment and possibly on human health, since no hazardous chemicals are added as compared to fungicide use. In addition, it is less expensive when compared to other sanitizing techniques after the initial purchase of the equipment.

3.4. Experiment 2

3.4.1. Efficacy of electrolyzed water treatment SAI-EW and AEW against *B. cinerea*

The results obtained showed that electrolyzed water treatment (SAI-EW and AEW) concentration, dipping duration and change in storage temperature had a significant effect ($p \leq 0.05$) on the lesion area of *B. cinerea* (Figure 3.5). At the end of storage at 15 °C, for SAI-EW treated fruit, the lesion area doubled (50%) from the initial growth area for EW-1 (Figure 3.5B). A significantly smaller lesion area was observed with concentrations 200 mg L^{-1} and 300 mg L^{-1} and the curative efficacy was most effective at these concentrations. Furthermore, varying dipping duration at low concentrations (EW-1) had a significant difference in lesion area. The highest lesion area with *B. cinerea* growth were observed for non-treated control and 100 mg L^{-1} for 10 min (EW-1) treated fruit stored 15 °C (Figure 3.5 A). From the fruit treated with AEW, the highest growth of *B. cinerea* was for 100 mg L^{-1} for 10 min (EW-1a) after 9 days of storage at 15°C and the lesion area increased > 36%, > 84%, and 93% throughout the storage duration (Figure 3.8). This study showed that SAI-EW concentration at 100 mg L^{-1} was not effective as a curative strategy against *B. cinerea.* However, comparing the results of the lowest SAI-EW concentration (100 mg L^{-1}) to the control non-treated apples at the end of storage, the growth of *B. cinerea* with samples under control apple sample were 85% higher. Furthermore, comparing the results of the lowest AEW concentration (100 mg L^{-1}) to the control non-treated apples at the end of storage, the growth of *B. cinerea* with samples treated with AEW under control apple sample was 99% higher. In a similar study, Yousef *et al*. (2018) demonstrated the effect of alkaline and acidic electrolysed water against grey mould (*B. cinerea*) on table grapes. The authors found that the acidic electrolysed water was the most effective in reducing *B. cinerea* by 98%, as compared to alkaline electrolysed water that reduced *B. cinerea* by 77%, respectively.

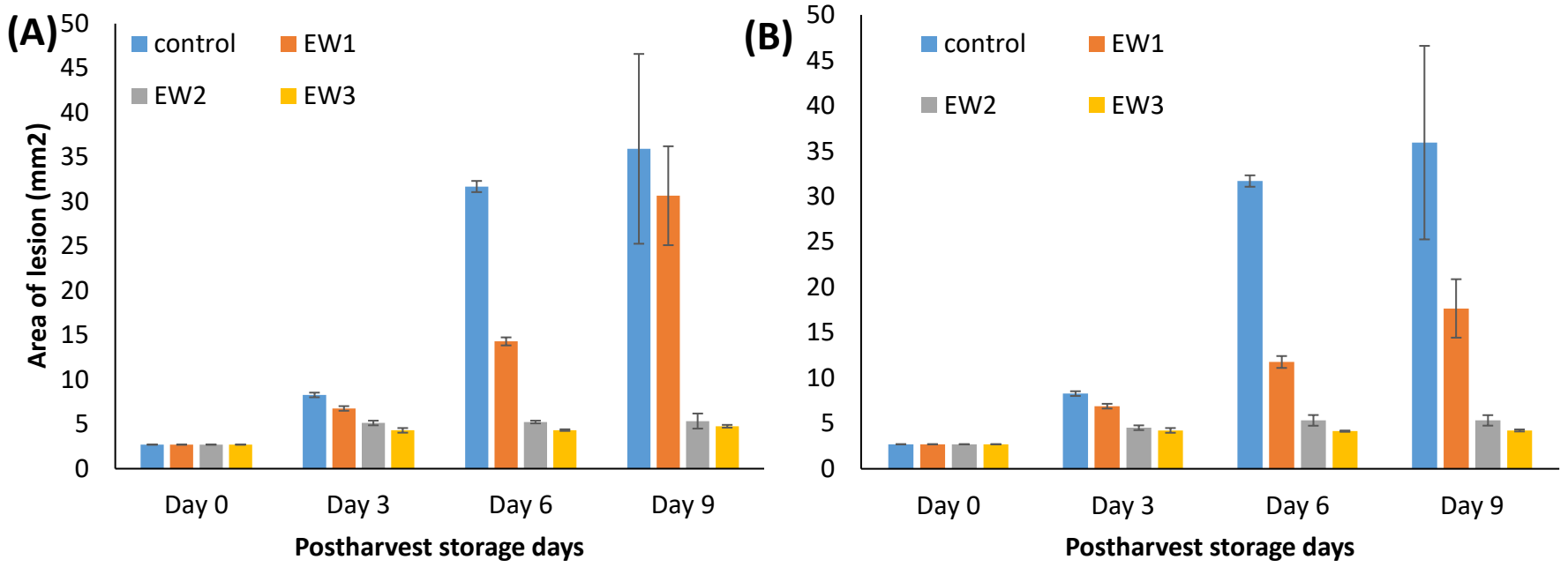

Figure 3.5. (A) Effects of SAI-EW concentrations, 10 min dipping duration and storage duration on the lesion zone (mm^2) of apples inoculated with *B. cinerea* during storage at 15°C for 9 days **(B)** Effects of SAI-EW concentrations, 15 min dipping duration and storage duration on the lesion zone (mm^2) of apples inoculated with *B. cinerea* during storage at 15°C for 9 days.

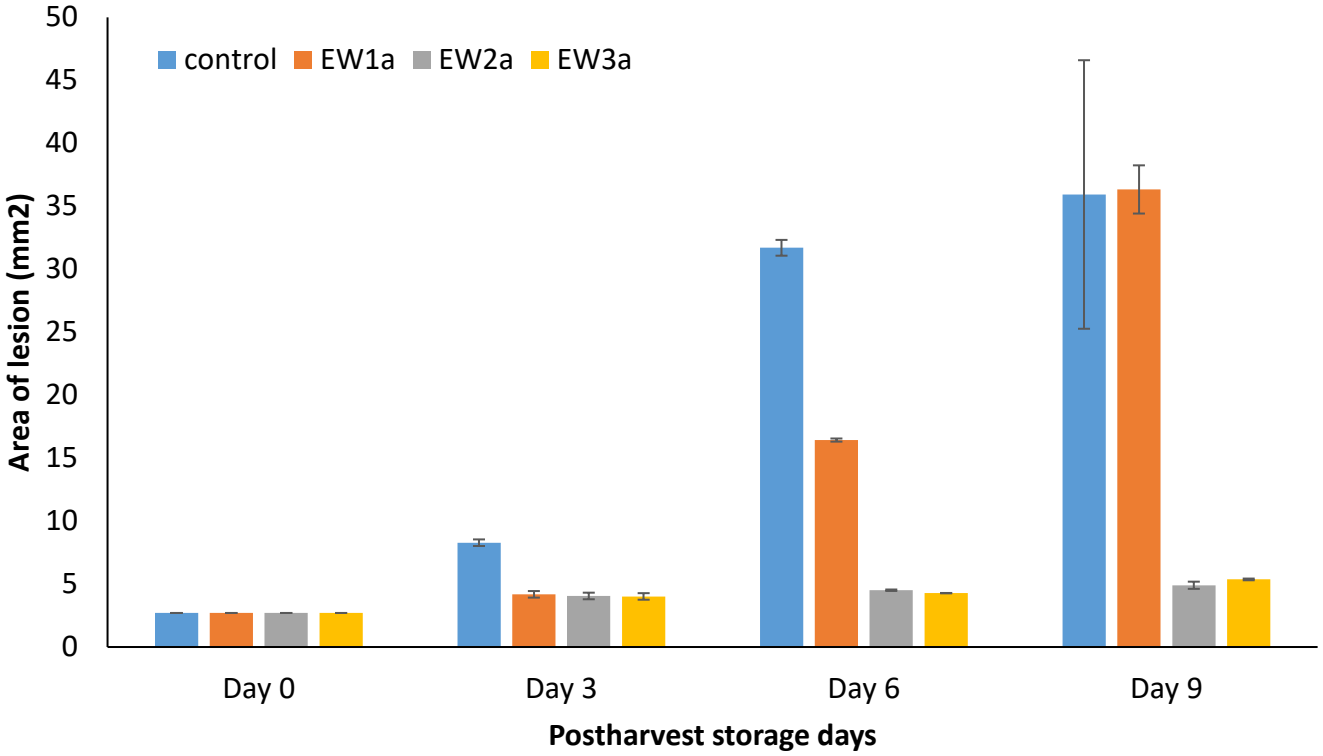

Figure 3.6. Effect of AEW concentrations, 10 min dipping duration and storage duration on the lesion zone (mm^2) of apples inoculated with *B. cinerea* during storage at 15°C for 9 days.

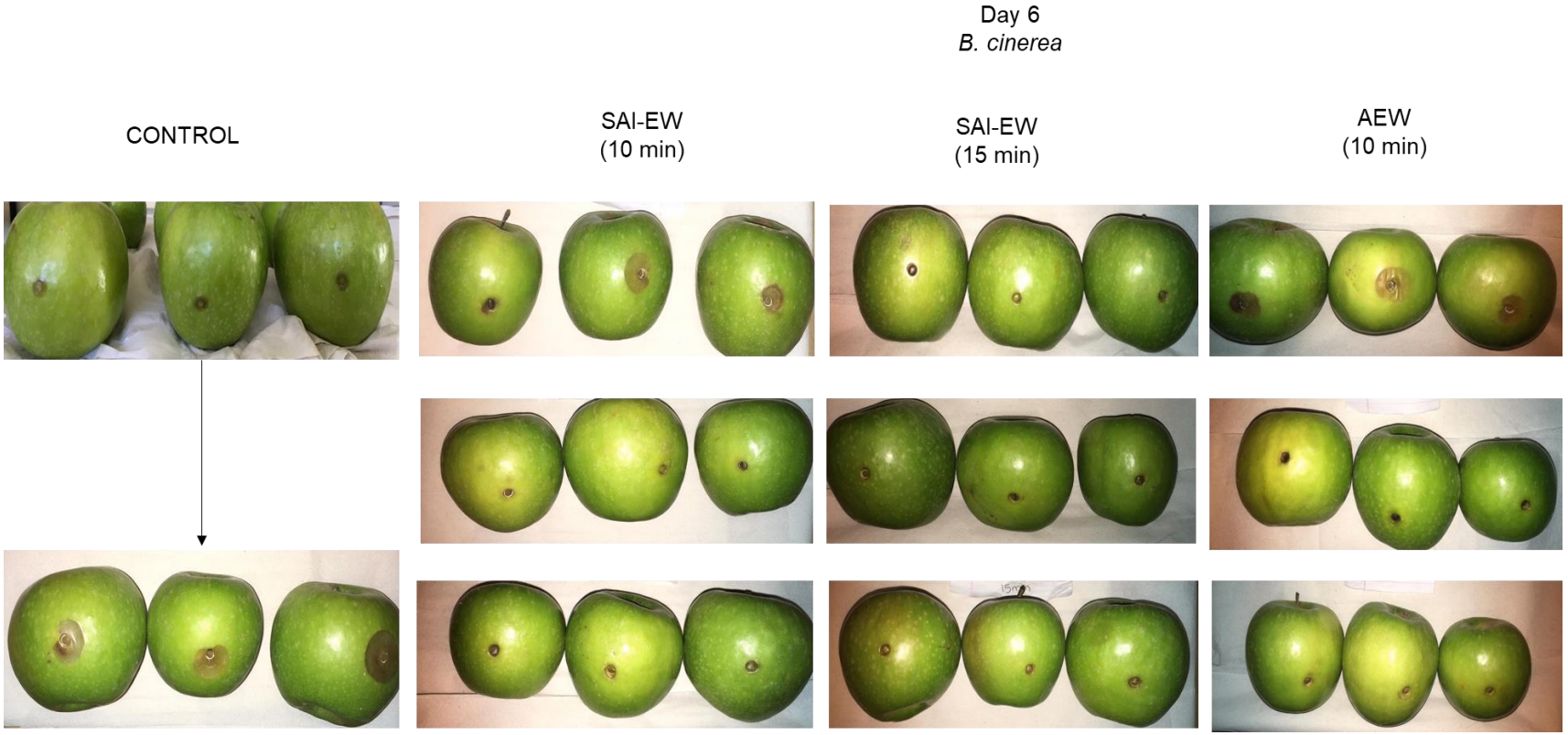

Figure 3.7. Differences in lesion area/ growth of *B. cinerea* on 'Granny Smith' apple at day 0 and after 6 days due to the effect of different electrolyte water treatment at 15 °C.

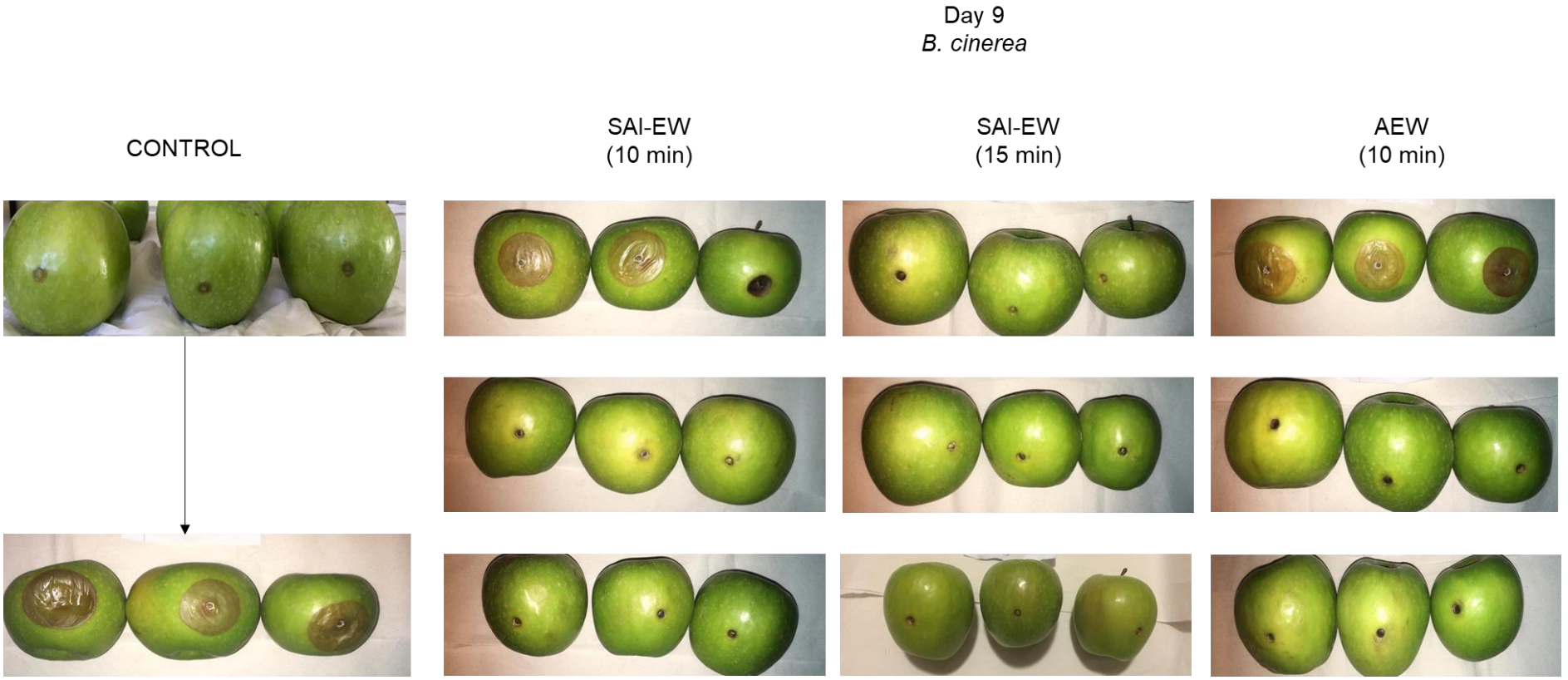

Figure 3.8. Differences in lesion area/ growth of *B. cinerea* on 'Granny Smith' apple at day 0 and after 9 days due to the effect of different electrolyte water treatment at 15 °C.

3.4.2. Efficacy of electrolyzed water treatment SAIEW and AEW against *P. expansum*

The results obtained showed that electrolyzed water treatment (SAI-EW and AEW) concentration, dipping duration and change in storage temperature had a significant effect ($p \leq 0.05$) on the lesion area of *P. expansum* (Figure 3.9). At the end of storage at 15 °C, for SAI-EW and AEW treated fruit, the lesion area was >79% and >65% respectively, compared to the initial growth area for EW-1 (Figure 3.9 A). A significantly smaller lesion area was observed with EW-2, EW-3, EW-2a and EW-3a (concentrations 200 mg L^{-1} and 300 mg L^{-1}) for SAI-EW and AEW respectively and the curative efficacy was most effective at these concentrations (Figure 3.9 A, B). Furthermore, varying dipping duration at low concentrations (EW-1, EW-1a) had a significant difference in lesion area (Table 3.4). At day 6 of storage for SAI-EW treated fruit, the lesion growth doubled for EW-1 and the control as compared to day 3 postharvest storage days (Figure 3.9 A). The highest lesion area with *P. expansum* growth were observed for non-treated control and 100 mg L^{-1} for 10 min (EW-1) SAI-EW treated fruit stored 15 °C (Figure 3,9 A). From the fruit treated with AEW, the highest growth of *B. cinerea* was for 100 mg L^{-1} for 10 min (EW-1a) after 9 days of storage at 15°C and the lesion area increased > 0.13%, >63%, and 65% throughout the storage duration (Figure 3.9 B). This study showed that SAI-EW concentration at 100 mg L^{-1} was not effective as a curative strategy against *B. cinerea.* However, comparing the results of the lowest SAI-EW concentration (100 mg L^{-1}) to the results of the lowest AEW concentration and the control non-treated apples at the end of storage, AEW was more effective in inhibiting the growth of *P. expansum*.

Youssef and Hussien, (2020), assessed the efficacy of alkaline and acidic electrolysed water against *P. digitatum* and *P. italicum* on 'Valencia' oranges. The authors found that both the components of the electrolysed water exhibited decontaminating activity against the two tested *Penicillium* species, however with a clear strong effect for the acidic component. In another study, Hussien *et al.* (2017) investigated the effect of alkaline (AlEW) and acidic (AEW) electrolysed water to control *Penicillium* species of citrus. The authors found that higher levels in AEW and AlEW (500 -1000 mg L^{-1}) showed the strongest biocidal effect on *Penicillium spp.* This study further demonstrated that using the optimum level of electrolysed water, AEW achieved higher inhibition for *P. digitatum, P. italicum* and *P. ulaiense* and the percentage of reduction of decay incidence due to AlEW was 20, 20 and 27% for green, blue and whisker moulds, respectively. In case of AEW, the percentage of reduction was 27, 33 and 33% for green, blue and whisker moulds, respectively as compared to water control. Previous studies have suggested the higher biocidal effect of acidic electrolysed water,and suggested alkaline electrolysed water to be used in cleaning and decreasing before the application of acidic electrolysed water (Koseki and Itoh, 2000; Ayebah and Hung, 2005). Therefore, the results of the present research showed that AEW was more effective than (SAI-EW). However, the

overall study confirms that electrolysed water is an effective treatment and has a good control over *P. expansum* on ‘Granny Smith’ apples.

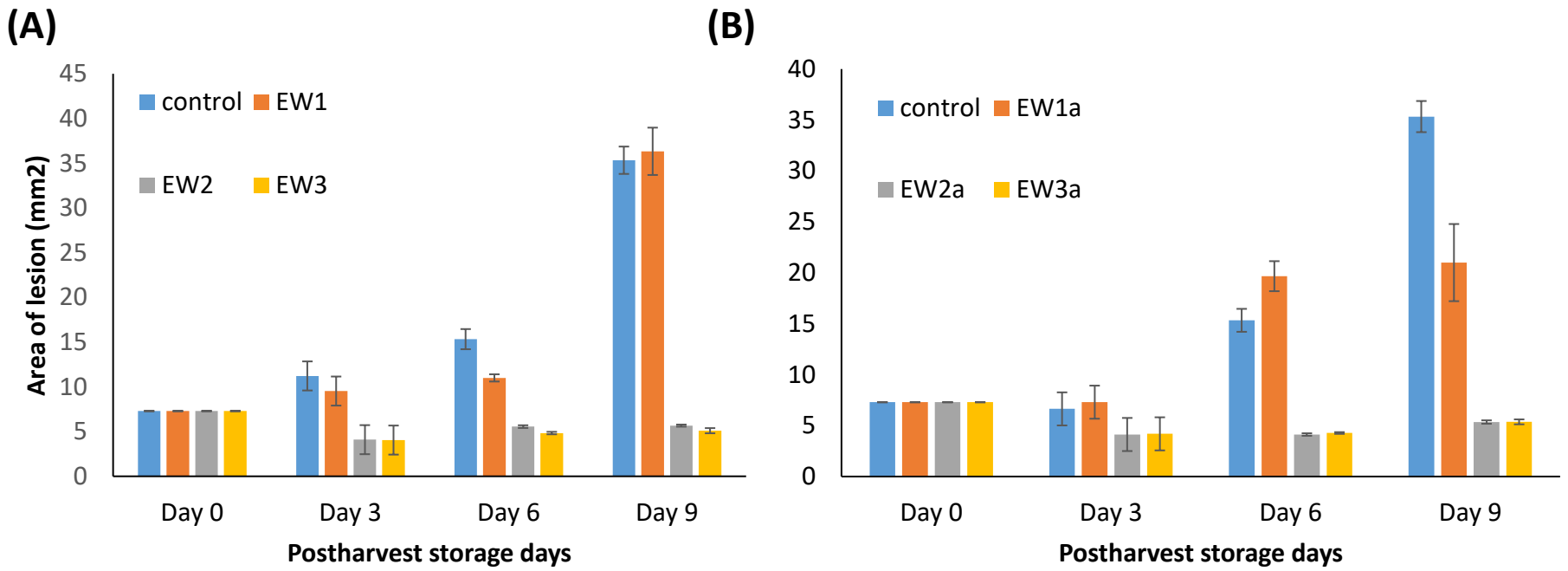

Figure 3.9. (A) Effects of SAI-EW concentrations, and storage duration on the lesion zone (mm^2) of apples inoculated with *P. expansum* during storage at 15°C for 9 days **(B)** Effects of AEW concentrations, and storage duration on the lesion zone (mm^2) of apples inoculated with *P. expansum* during storage at 15°C for 9 days.

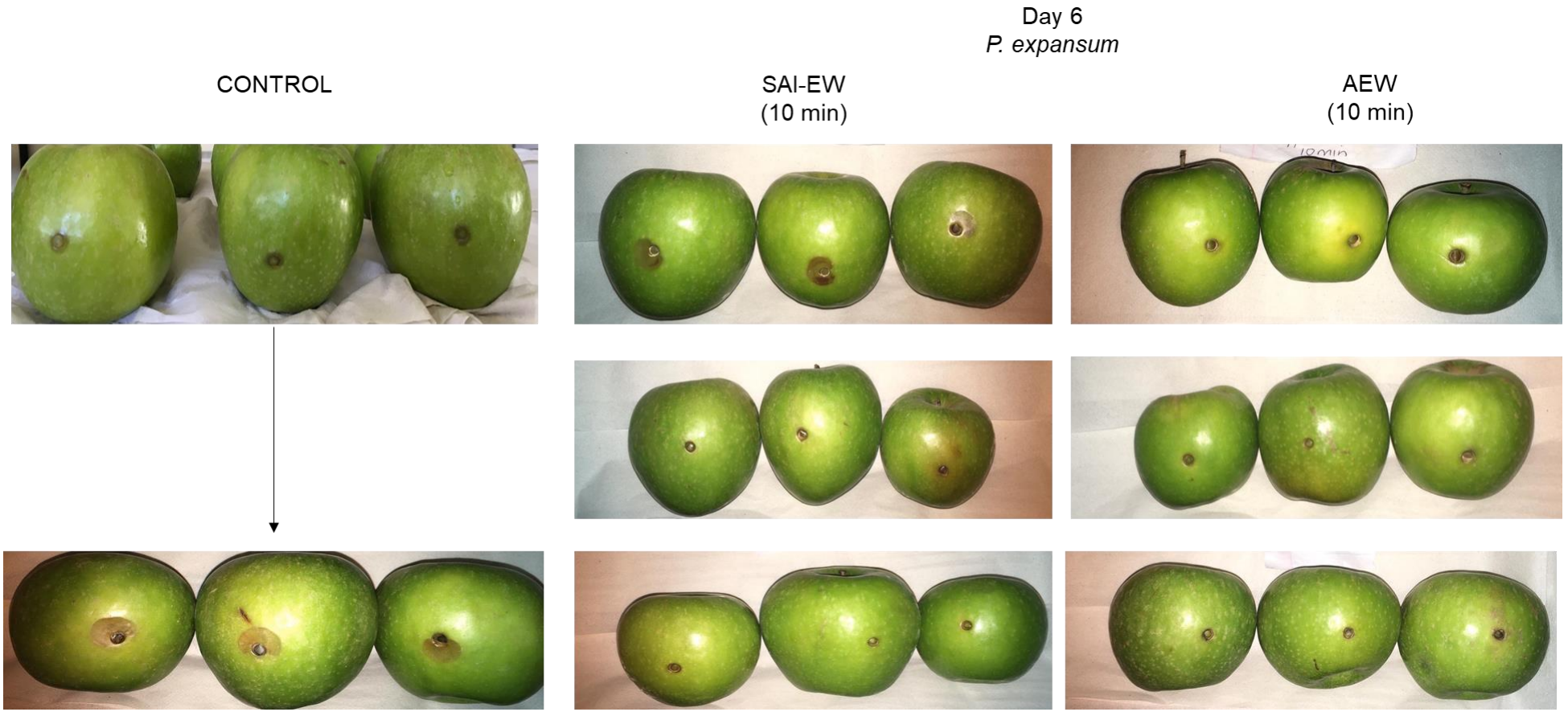

Figure 3.10. Differences in lesion area/ growth of *P. expansum* on 'Granny Smith' apple at day 0 and after 6 days due to the effect of different electrolyte water treatment at 15 °C.

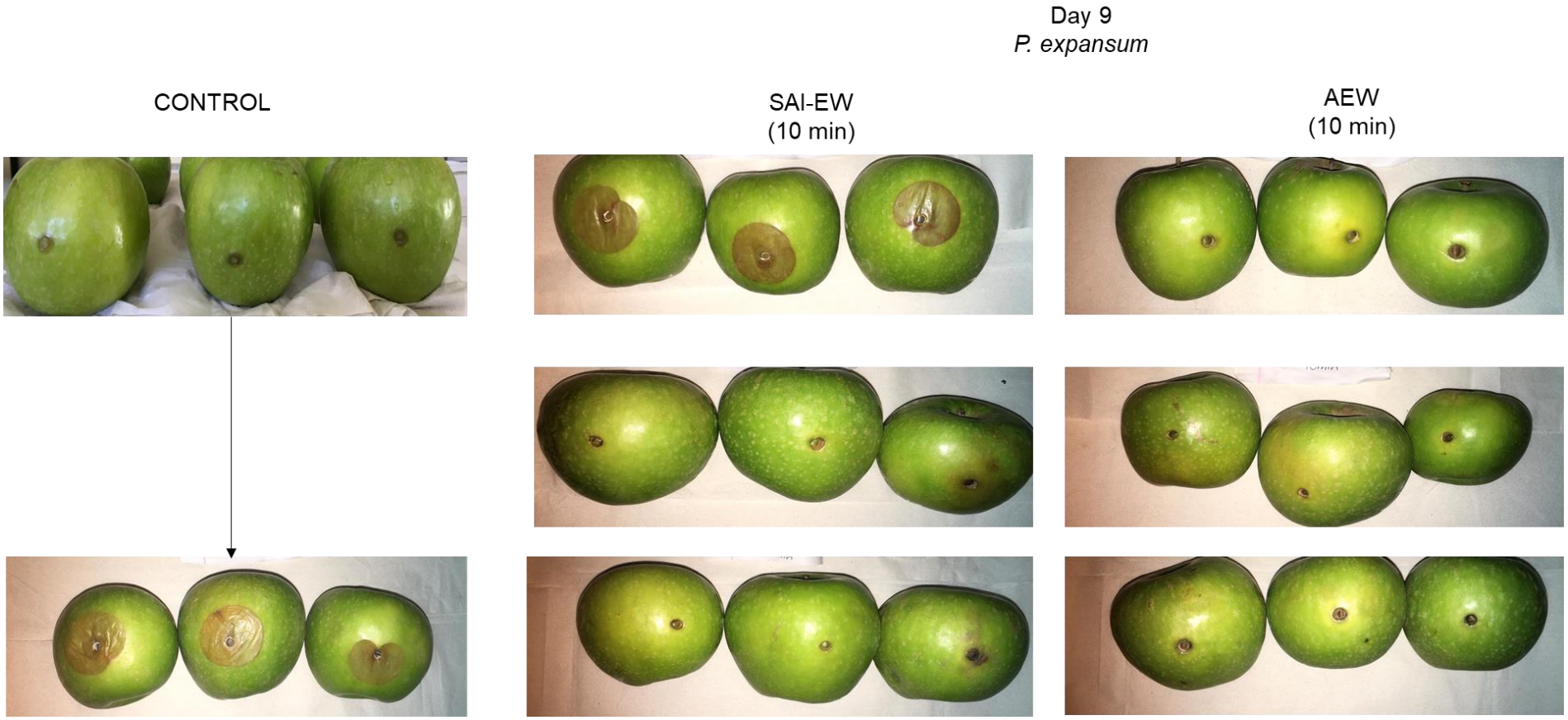

Figure 3. 11. Differences in lesion area/ growth of *P. expansum* on 'Granny Smith' apple at day 0 and after 9 days due to the effect of different electrolyte water treatment at 15 °C.

3.5. Conclusion

Experiment 1 explored alternative postharvest pre-treatment strategies using slightly alkaline electrolyzed water (SAI-EW) treatment for maintaining quality and managing decay of 'Granny Smith' apples. Overall, the 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 concentrations 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 (10 and 15 min) 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.* Using higher concentrations of EW above 300 mg L^{-1} showed adverse effects on lenticels. Experiment 2 explored a better curative efficacy using slightly alkaline (SAI-EW) and acidic electrolyzed (AEW) water treatments against *B. cinerea* and *P. expansum*. Overall, both SAI-EW and AEW had significant curative efficacy against the growth of *B. cinerea* and *P. expansum*. However, based on the outcomes of this study, dipping 'Granny Smith' apples in SAI-EW and AEW (100 mg L^{-1}) was not effective. The curative efficacy of the electrolyzed water treatments was AEW> SAI-EW. In addition, the findings demonstrate the potential of both SAI-EW and AEW electrolyzed water as an alternative fruit decay management strategy. Further studies are required to elucidate the impact of this treatment on nutritional, sensorial and functional properties of 'Granny Smith' apples.

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CHAPTER 4

Impact of alkaline electrolyzed water treatments on the physicochemical, phytochemical, antioxidant properties and natural microbial load on 'Granny Smith' apples during storage

Abstract

Providing consumers with safe fruits can greatly increase their recommended intake of vitamin A, vitamin C, folate, minerals, dietary fibre, and phytonutrients per day, so as to improve their health. 'Granny Smith' apples are popular in South Africa due to their high nutritional value and desirable taste. However, 'Granny Smith' apples are susceptible to various quality deterioration and microbial decay during post-harvest. Therefore, post-harvest technologies that control and maintain the microbial quality and nutritional value of fresh fruit need to be developed. In the present study, fresh apple cv. "Granny Smith" were treated with alkaline electrolyzed water (AIEW) (200 and 300 mg L^{-1}) under varying dipping durations (10 and 15 min) for the inactivation of microflora (total plate count, yeast and mould). Additionally, total phenolics, flavonoids, flavonols, antioxidant activity, pH, titratable acidity, Brix and colour values influenced by electrolyzed water (AIEW) were investigated. These were then compared to sodium hypochlorite (NaOCl, 200 mg L^{-1}) and non-treated (control). Apples treated with AIEW maintained low pH, titratable acidity (TA) and total soluble solids (TSS) compared to other treatments ($p > 0.05$). The interaction of treatments and storage duration had a significant impact on total polyphenols and total flavonoids ($p \leq 0.05$). At the end of storage day 21, AIEW treated apples better maintained the antioxidant capacity compared to control ($p \leq 0.05$). Treatments with AIEW and sodium hypochlorite had no effect on scald incidence. Treatments with SAI-EW resulted in ≈ 2 Log reduction in total aerobic mesophilic bacteria (from 4.1 Log CFU cm^2 to 2.2 Log CFU cm^{-2}) and < 1 Log reduction for yeast and mould (from 3.9 Log CFU cm^2 to 2.7 Log CFU cm^2) count. At the end of storage, AIEW treated apples (with 200 mg L^{-1} for 15 min; EW4) maintained the lowest total aerobic mesophilic bacteria and yeast and mould count compared to the control samples. Overall results of this study demonstrated the efficacy and potential of electrolyzed water treatment as an alternative to conventional industry/pack-house practice (sodium hypochlorite treatment) for apple fruit.

Keywords: Deciduous fruit, total polyphenols, antioxidant capacity, *Malus domestica*

4.1. Introduction

Apple (*Malus domestica*) fruit, a renowned deciduous fruit, represents one of the most nutritional fruits. It is produced and consumed extensively owing to its taste, nutritional content and economic value. In addition, it is rich in compounds including dietary fibre, total soluble solids (TSS), titratable acidity, polyphenols, flavonoids and antioxidant activity (Lee *et al.*, 2003; Duda-Chodak *et al.*, 2011; Büchner, 2015; Wang *et al.*, 2015; Musacchi and Serra, 2018). Therefore, apples are a good source of the phenolic compounds and antioxidants for humans (Wolfe *et al.*, 2003). Previous studies indicate that the apple fruit possess many biological activities which increase the phenolic compounds and antioxidants in apples, in turn, which may help to reduce the risk of developing cancer, hypertension, diabetes, and heart disease, implying apple fruit have a great potential to contribute towards human health (Eberhardt *et al.*, 2000; Boyer and Liu, 2004; Tsao, 2015). Thus, apple fruit is well-liked for people and plays a considerable role in the global fruit market.

According to DAFF (2020) during the 2019/20 season, apples contributed approximately 33%, which is 6.1 billion of the total 18.2 billion gross value contributed by deciduous fruits in South Africa. However, apple fruit is highly perishable and susceptible to various post-harvest diseases, which lead to loss of quality during storage. The postharvest losses of apples can range between 25-28%, with an additional loss occurring before harvest because of premature drop of fruit in orchards (Tarabay *et al*., 2018; van der Walt *et al*., 2010). Chlorine is the commonly used decontamination agent in the food industry (Aryal and Muriana, 2019). However, the reactivity of the chlorine species present in chlorine-based sanitizers could be different than compared to other chlorine-based sanitizers because of the reactive and complex nature of chlorine. In addition, the produce industry has raised concerns regarding the additional regulatory barriers, limitations and safety, and regulations for the use of chlorine in its present form (Ayebah and Hung, 2005; Tucker and Featherstone, 2010; Nan *et al.*, 2019). For this reason, it is necessary to develop a novel, safe, environmentally- friendly, and low-cost preservation technique for apple fruit.

Electrolyzed water (EW) is considered as one potential substitute for traditional chlorine treatment. It is an aqueous solution with special physical and chemical properties that is produced by a low- voltage direct current electrolysis of a dilute sodium chlorine solution in a special device, which can change its available chlorine concentration (ACC), pH value, and oxidation reduction potential (Al-Haq *et al.*, 2005; Huang *et al.*, 2008; Chen *et al.*, 2017, 2019b). On the anode side, acidic electrolysed water (AEW) is produced, with high dissolved oxygen, free chlorine and is characterized by a low pH (2- 3) and a high oxidation-reduction potential (ORP > 1000 mV) (Huang *et al.*, 2008). At the same time, on the cathode side, alkaline electrolysed water (AIE) is produced, with high pH (11.0-13), highly dissolved

hydrogen and low ORP (-795 to -900 mV) (Huang *et al.*, 2006), and neutral water (NEW) (pH 7.0 to 8.0, ORP 750 mV) (Rahman *et al.*, 2016). Electrolyzed water is not harmful to human health as compared to chlorine. When electrolyzed water comes into contact with organic matter or is diluted with tap water, it is converted back to ordinary water (Aday, 2016). In addition, electrolyzed water is convenient to prepare, and its application cost is inexpensive (Huang *et al.*, 2008).

The post-harvest application of EW has been investigated in controlling microbial contaminations in apple fruit (Kim *et al.*, 2000; Okull and LaBorde, 2004a,b; Hung *et al.*, 2010; Graça *et al.*, 2011; Kim and Hung, 2014; Ferri *et al.*, 2016; Chen *et al.*, 2019). For instance, Okull and LaBorde, (2004b) investigated the activity of electrolyzed oxidizing water against *Penicillium expansum* in suspension on wounded apples. The treatment decreased viable spore populations by greater than 4 and 2 log units, respectively. Nimitkeatkai and Kim (2009) observed the effect of EW on washing apples using a strong acidic EW with pH 2.8 and slightly acidic EW with pH 6.5. The authors observed improved sensory quality for both strong and weak acid EW (either 2 or 5 min). 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 in the concentration of some pesticide residues on the fruit. In another study by Graça *et al.*, (2010) neutral electrolyzed water (NEW) and acidic electrolyzed water (AEW) were used to inactivate food-borne pathogens on the surface of fresh cut apples, pears, and oranges. From this study emerged that both (NEW and AEW) were able to reduce the microbial populations and that AEW specifically had a higher efficacy (Graça *et al.*, 2011). Kim and Hung (2014) studied the effect of electrolyzed water on enzymatic browning in 'Red Delicious' apples. This study demonstrated that alkaline electrolysed water can be a promising treatment solution to prevent browning and thereby maintain the quality of fresh-cut apples.

Moreover, Chen *et al.* (2020) investigated the use of acidic electrolyzed water (AEW) to treat longan fruit and evaluate the effects of AEW treatment on storability, quality attributes and nutritive properties of longan fruit during storage. This study indicated that EW treatment could suppress the decrease of chromaticity values of L*, a* and b* of the fruit surface, keep higher amounts of pericarp carotenoid, chlorophyll, flavonoid and anthocyanin, maintain higher amounts of pulp total soluble solid (TSS), total soluble sugars, sucrose and vitamin C. In another study by (Li *et al.*, 2020a), changes in the number and species of microorganisms and levels of anthocyanins, total phenolic, and antioxidants in eggplants treated with acid electrolyzed water (AEW), slightly acid electrolyzed water (SAEW), and sterile distilled water (DW) were examined. The authors observed that there was an increase of 1.22 and1.76 log CFU/g, and 1.51 and 1.92 log CFU/g counts of total aerobic bacteria, and yeast and mould in eggplants treated with DW than those treated with SAEW and AEW, respectively and that the

anthocyanin content in samples treated with AEW and SAEW was higher than that detected in samples treated with DW. Furthermore, that the abilities of the eggplants treated with AEW and SAEW to scavenge DPPH were higher than those of eggplant samples treated with DW. In another study by Soquetta *et al*. (2019), the authors evaluated the use of acidic electrolyzed water, basic electrolyzed water, and slightly acidic electrolyzed water (AEW, BEW, and SAEW, respectively) as solvents in ultrasound (US) to extract bioactive compounds (total phenolic (TP), total flavonoids (TF), and antioxidant capacity (FRAP)) from citrus fruits. The authors observed that the extract obtained with US + SAEW presented the highest values for TP (4,324.32 mg GAE.100 g^{-1}) of tangerine peel and FRAP (663.63 µmol TEAC.100 g^{-1} of tangerine peel) and the highest TF content was found for US + AEW (691.76 mg EQ.100 g^{-1} of tangerine peel).

Currently, there is very limited information on the impacts of varying EW concentrations and dipping duration on the phytonutrients of 'Granny Smith' apples. Thus, the set hypothesis for this study was that varying the concentration of EW and dipping duration would enhance antioxidant properties, boost accumulation of polyphenols and flavonoids, maintain physicochemical attributes and reduce surface microbial load on 'Granny Smith' apples. The main objectives of this study were to evaluate the: (a) effects of electrolyzed water treatments in removing/reducing aerobic mesophilic bacteria and yeasts and moulds on the surface of the apple fruit, (b) impacts of EW on fruit texture, changes in colour, total soluble solids (TSS), titratable acidity (TA), and phytonutrients (antioxidant activity, polyphenol content, total flavanol and total flavonol content) of 'Granny Smith' apples and (c) effects of electrolysed water treatments on superficial scald incidence of 'Granny Smith' apples.

4.2. Materials and Methods

4.2.1. Plant material

Fresh 'Granny Smith' apples (*Malus domestica*) were harvested at commercial maturity from the Agricultural Research Council (ARC) Elgin Research Farm, Grabouw, South Africa. Harvested fruit were transported in cool trucks 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 a regular atmosphere (RA) at 0.5°C for 3 months prior to investigation. Apples were not treated with ethylene inhibitors to mimic packhouse short-term sales practices.

4.2.2. Electrolyzed water preparation

In this study, the electrolyzed water was generated using the ELA-12 000ANW system (ECA Technologies, Envirolyte, South Africa). The AIEW was generated by electrolysis of hydrochloric acid in the range (0.05 %), sodium chloride (0.26 %) and water (99.69 %). The electrolyte flow passed through an electrolytic cell at a rate of 2 mL min^{-1}, at a setting current of 3.8–3.9 V and amperage 10 A. The pH and ORP were measured immediately after preparation and right before experiments to confirm that they were not significantly changed using pH meter (D-22, Horiba, Kyoto, Japan) and ORP meter (HM-60V, TOA Electronics Ltd., Tokyo, Japan), respectively. The AIEW obtained consisted of: available chlorine concentration (ACC) of 500 mg L-1, ORP > –800 mV, and pH = 11-13. Food grade sodium hypochlorite (NaOCl, 11.5% M/V) solution was purchased from Protea Chemicals, Sandton, South Africa**.** AIEW solutions were diluted to desired concentrations (200–300 mg L^{-1}) prior to dipping the apples. The dipping duration selected was based on the average pack-house processing time for apples. Description of treatments and their abbreviations as used further in this study are presented in Table 4.1. After treatments, all the samples were stored at 5 °C and 90 ± 2% RH for 21 d and analyses were conducted in triplicate on days 0, 7, 14, and 21.

Table 4.1. Description of treatments and abbreviations used in the study.

Treatment(s)			**Treatment(s) abbreviation**
Active compound	**Concentration (ppm)**	**Dipping duration (min)**	
NaOCl	200	10	C1
NaOCl	200	15	C2
AIEW	300	10	EW1
AIEW	300	15	EW2
AIEW	200	10	EW3
AIEW	200	15	EW4
Non-treated	-	-	Control

4.2.3. Physicochemical properties

4.2.3.1. Texture profile

Tissue strength of individual apples exposed to different treatments and non-treated controls were measured using a texture analyser (FTA 20, Güss, South Africa) according to a method described by Nsumpi, Belay, and Caleb (2020). Two opposite sides (left, right) of the fruit were gently peeled, each was placed on the texture analyzer 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. All measurements were conducted in triplicate (n = 3) per treatment and tissue strength was expressed in Newton (N)(Pathak *et al*., 2017).

4.2.3.2. Colour

Colour changes on each apple fruit were measured based on the Commission International del' Eclairage (CIE) colour system using a digital Chroma-meter (CR 400/410 Konica Minolta Sensing Inc., Japan). Measurements were taken from opposite sides of 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. Chroma (C*) and $h°$ angles were calculated (Belay *et al*., 2017).

4.2.3.3. Total soluble solids (TSS), pH and titratable acidity (TA)

Apples were processed into juice for each treatment using a juice extractor (4294 J700, Braun, China). Total soluble solids content (TSS) of the juice was measured using a calibrated hand-held refractometer (Pal-1, Atago Co. Ltd., Japan). Titratable acidity (TA) of the juice samples was measured using a 60 mL juice, titrated against 0.333 N sodium hydroxide (NaOH) to a pH point of 8.2, using Crison Titromatic 1S/2B (Crison Instruments, Barcelona, Spain). The 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 before they were centrifuged at 2951 x g using the Hermle Z206A compact centrifuge (Wehingen, Germany). The supernatants were then used for the phytochemical and antioxidant evaluations.

4.2.4. Analyses of total phenolics, flavonoids, and total flavanol content

4.2.4.1. Determination of total phenolic content

The total phenolic concentration (TPC) was measured using the Folin-Ciocalteu method as described by (Belay, Caleb, & Opara, 2017). Briefly, 125 µL of the Folin-Ciocalteu

reagent (diluted 10x with distilled water) was added to the 25 µL apple juice sample in each clear plate well. After 5 min, 100 µL of 7.5% sodium carbonate (Na_2CO_3) was added into each well. The plate was allowed to stand for 2 h at room temperature in the dark before taking a reading. Absorbance was read at 750 nm using a microplate spectrophotometer (Fluostar Omega, BMG Labtech, Offenburg, Germany). Gallic acid equivalent (GAE) standard curve was used to extrapolate the total phenolic content (TPC) and was expressed as mg GAE L^{-1}.

4.2.4.2. Determination of total flavonol content

The total flavonol content was determined according to a slightly modified method described by Pavun, Uskoković-Marković, Jelikić-Stankov, Dikanović, & Durdević, 2018). A 12.5 µL of HCl (0.1 %) in 95 % ethanol was added into 12.5 µL of apple juice in each clear plate well. Thereafter, 225 µL of HCl (2 %) was added to each plate well. The plate was left for 30 min at room temperature before taking a reading. The absorbance was read at 450 nm using the microplate spectrophotometer (Fluostar Omega, BMG Labtech, Offenburg, Germany) against a standard curve of quercetin. The total flavonol content was expressed as mg quercetin equivalent (QE) L^{-1} apple juice.

4.2.4.3. Determination of total flavanol content (TFAC)

The TFAC was estimated using a modified p-dimethylaminocinnamaldehyde (p-DMACA) method (Wang *et al.*, 2015). A 250 µL of p-DMACA (0.1 % in 1 M HCl in MeOH) was added to the clear plate well containing 50 µL of apple juice sample, thereafter, the plate was left for 30 min at room temperature before taking the reading. The absorbance was read at 640 nm using the microplate spectrophotometer (Fluostar Omega, BMG Labtech, Offenburg, Germany). The TFAC was calculated from a calibration curve using catechin as the standard. The total flavanol was expressed as mg catechin equivalents (CE) L^{-1} apple juice.

4.2.5. Determination of antioxidant capacity

4.2.5.1. Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay utilizes the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid radical cation ($ABTS^+$) previously described by Duda-Chodak, Tarko andTuszyński (2011) was used. The $ABTS^+$ was freshly produced by a reaction of 7 mM ABTS with 140 mM $K_2S_2O_8$. The reaction mixture was kept in the dark at room temperature for 24 h before use. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was used to prepare a standard curve. Sample (25 µL) was added to the clear plate well, thereafter, 300 µL of ABTS mix was added and the plate was left for 30 min at room temperature before taking the reading.

Absorbance reading was taken at 734 nm using the microplate spectrophotometer. The antioxidant capacity was expressed as mg Trolox equivalent (TE) L^{-1} of apple juice.

4.2.5.2. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to Benzie and Strain, (1996). Briefly, the FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 0.1M HCl, and 20 mM iron (III) chloride hexahydrate solution. The FRAP reagent was freshly prepared and was warmed to 37 °C in a water bath prior to use. A 10 µL of juice sample and 300 µL of the FRAP reagent were added to each well of a plate. The plate was left for 30 min at room temperature before taking the absorbance at 593 nm using a microplate spectrophotometer. A standard curve was set up using vitamin C and the results were calculated as mg vitamin C equivalent (VitCE) L^{-1} apple juice**.**

4.2.5.3. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was carried out according to a method described by Thaipong*et al.,* (2006). Apple juice sample (12 µL) was added to a black micro-well plate, thereafter, 138 µL of 1 mM fluorescein solution in 75 mM phosphate buffer (pH 7.4) was added. This is followed by the addition of 50 µL of the 25 mg/ml 2,2'-Azobis (2-methylpropionamidine) dihydrochloride (APPH) solution prepared in a phosphate buffer to start the reaction. The reaction was for 2 hrs in the fluorescence spectrophotometer (Fluostar Omega, BMG Labtech, Offenburg, Germany) set up with excitation and emission wavelengths of 480 and 530 nm respectively. The ORAC value was extrapolated from the area under the curve of the Trolox standard curve and expressed as mg TE L^{-1} of apple juice.

4.2.5.4. Evaluation of superficial scald intensity (scald index)

Fruits affected by superficial scald were counted at the end of each storage period (0, 7, 14 and 21 days). Each fruit was inspected visually and the percentage area of the fruit surface affected (scald severity) with the disorder was recorded. The severity of the disorder was evaluated and graded according to an index used by Ramokonyane *et al.* (2016), based on the percentage surface area of fruit affected, resulting in the following categories: Grade 0 = no scald (0%), Grade 1 = Slight scald (1-25%), Grade 2 = Moderate scald (26-50%), Grade 3 = Severe scald (51-75%), Grade 4 = Extreme scald (76-100%). The inspection of fruit was done after each storage interval at day 0, 7, 14, and 21 for fruit stored at 5 °C.

4.2.6. 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 vortexed for 60 min to remove the microbial load on the fruit surface. Threefold 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 poured 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 units per square centimetre of surface (CFU cm^{-2}) was calculated and transformed to Log CFU cm^{-2}.

4.2.7. 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% confidence interval using Statistica Software (version 13, StatSoft Inc. TIBCO Software Inc., USA). Duncan multi-range test was used to determine the difference between means. Correlation analysis was performed using XLSTAT software version 2016.06.37085 (Microsoft, USA). Results were presented as mean ± standard deviation.

4.3. Results and discussion

4.3.1. Physicochemical parameters

4.3.1.1. Tissue strength

Based on statistical analysis, the interactions of treatments, dipping and storage duration had no significant impact ($p > 0.05$) on the textural profile of the apples (Table 4.2). 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), but was not significantly different from other treatments besides the non-treated (control) which had the lowest (5.13 ± 0.22 N) tissues strength (Table 4.2). In addition, no significant difference was found between chlorinated water and AIEW treated apples at the end of storage ($p > 0.05$). There are only a few studies in the literature regarding the impact of EW on the texture profile of 'Granny Smith' apples. This finding is in agreement with Ding *et al.* (2015) who found no significant difference in the firmness of 'Cherry' tomatoes treated with SAEW. Hung *et al.* (2010a) demonstrated in their study on strawberries, that washing treatment of electrolyzed water (EW) and chlorinated water did not lead to a decrease in the firmness of strawberries.

The firmness of fruit can be related to the mechanical properties of the plant cell wall

and internal pressure of the cells (i.e., the turgor pressure). Changes of cell wall biopolymers, in particular the pectin, may impair the cell wall structure and result in tissue softening (Harker *et al.*, 2006; Ahmadi-Afzadi *et al.*, 2013; Chen *et al.*, 2017). Furthermore, the epidermal layers of a mature apple fruit consist of three distinct layers: (i) the waxy layer (with partially crystalline character causing the glossy reflection), (ii) the cutin layer (consisting of polyhydroxy fatty acids) and (iii) the cell walls (Veraverbeke *et al.*,2001). Wax layer is believed to be the main barrier limiting mass transport into and from the fruit tissue (Baur *et al.*, 1996; Veraverbeke *et al.,* 2001). Hence, it is believed that the wax layer of the cuticle prevented wetting of the fruit tissue. The texture profile of fresh apples is one of the most critical quality attributes influencing consumer appeal and marketability (Harker *et al.*, 2008; Hussain *et al.*, 2012).

4.3.1.2. Colour

The colour parameters (*L**), (*a**) and (*b**) values were measured for the treated and non-treated 'Granny Smith' apples (Table 4.2). Parameter *L** value represents the brightness of colour (Chen *et al.*, 2020). 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 ($p > 0.05$). On the other hand, the control samples, which had an initial increase in *L** values declined significantly on day 21 ($p \leq 0.05$). In addition, on day 21, the highest *L** (56.2 ± 2.13) value was observed for apples dipped for 15 min in 300 mg L^{-1} AIEW (EW1), while control samples had the least *L** (48.0 ± 2.42) ($p \leq 0.05$). Overall, treatments did not significantly change the chromaticity *L**, but maintained higher values than the control (Table 4.2). A similar observation was reported by Chen *et al.* (2020) for longan fruits.

As shown in Table 4.2, the chromaticity values *a** and *b** for all the treatment groups were not significantly affected by the interaction of treatments and storage days ($p > 0.05$). A negative decline in *a** values from -20.09 to a range of -4.4 to -14.9 was observed across all samples on day 0 to 21 ($p \leq 0.05$), while *b** values increased slightly during storage, it did not change significantly ($p > 0.05$) amongst the treatment. However, at the end of storage day 21, the lowest *a** value (-4.44 ± 1.17) was found in the control samples, while other treated samples were between -13.7 to -15.1. Similarly, the lowest *b** value (28.5 ± 3.92) was observed in the control samples (Table 4.2). Similarly, other studies have found no significant impact of EW application on measured colour attributes for other fresh produce before and after treatment (Jemni *et al.*, 2014; Youssef and Hussien, 2020). Apple peel colour formation is influenced by various pigmentation such as chlorophyll and carotenoids located in plastids as well as the phenolic pigments such as anthocyanins located in the vacuole (Musacchi and Serra, 2018). Colour reflects the maturity and freshness quality of the fruit (Gao *et al.,* 2019;

Poirier, Mattheis, & Rudell, 2020). Therefore, maintenance of colour is vital during postharvest handling and storage to ensure marketability and consumer acceptance.

4.3.1.3. Total soluble solids (TSS), titratable acidity (TA) and pH

Effects of EW and chlorine treatments and storage duration on the total soluble solids content of 'Granny Smith' apples are summarized in Table 4.2. This quality attribute (TSS) fluctuated throughout the study and statistical analyses showed that interactions of treatment types and storage duration had no significant ($p > 0.05$) effect. However, at a given storage time TSS values obtained for treated and non-treated apples were found to be significantly different ($p \leq 0.05$). The TSS content of fruit increased for control (13.6 °Brix), EW3 (13.0 °Brix, SAI-EW, 200 mg L^{-1} at 10 min) and EW4 (13.3 °Brix, AIEW, 200 mg L^{-1} at 15 min) on day 7 and thereafter declined to 12.5, 12.2 and 12.4 °Brix, respectively, at the end of storage (Table 4.2). Control and EW4 samples had significantly higher TSS values on day 7 ($p \leq 0.05$), in comparison to other treatments. The observed fluctuation and decline in TSS could be attributed to intrinsic variation in the fruit metabolic process (e.g., respiration) at the different intervals (Chen *et al*., 2020). Total soluble solid content is a key component associated with apple fruit sweetness, and compared with the control fruit, AIEW treatment could maintain the TSS in 'Granny Smith' apples.

On the other hand, the interaction of treatment and storage duration had a significant effect ($p \leq 0.05$) on the TA content of apples. A continuous and significant decline in TA was observed across all treatments and control until the end of the experiment (Table 4.2). In contrast, Chen *et al*. (2020) reported a continuous increase in TA content (%) for longans treated with acidic EW. At the end of storage lowest TA content (0.68 g 100 mL^{-1}) was observed in apple fruit treated with chlorinated water dipped for 15 min, while the highest TA (0.86 g 100 mL^{-1}) was observed in the control apple fruit. Compared to the control samples at the end of storage, treatments EW1, EW2, EW3 and EW4 did not have significant impact. Furthermore, the pH value fluctuated during the storage period. An initial decline was observed on day 7 followed by a significant ($p \leq 0.05$) increase in pH across all the treatments and control on day 14 (Table 4.2). At the end of day 21, apples treated with chlorinated water had the lowest pH and AIEW treated apples did not differ significantly from initial reading. These results are consistent with other previous studies that have shown that using EW did not have considerable impact on both TA and pH (Hung *et al*., 2010a; Hung, Tilly andKim, 2010b; Bessi *et al.*, 2014).

Table 4. 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)	Firmness (*N*)	*Colour parameters*			TSS (° Brix)	TA (g 100 mL)	pH
			*L**	*a**	*b**			
C1	0	5.5 ± 0.35 ^{ab}	50.1 ± 6.87 ^{abc}	-20.1 ± 5.44	37.9 ± 2.31	12,66±0,31	1,23±0,03	3,18±0,01
	7	5.7 ± 0.22 ^{a}	53.1 ± 2.28 ^{ab}	-14.5 ± 2.43	38.1 ± 1.99	12,57 ± 0,42	1,11 ± 0,03	3,16±0,02
	14	5.8 ± 0.41 ^{ab}	51.8 ± 1.61 ^{bc}	-10.2 ± 2.09	36.1 ± 1.92	12,8 ± 0,20	0,89±0,11	3,17±0,06
	21	5.5 ± 0.21 ^{ab}	53.9 ± 2.89 ^{ab}	-14.1 ± 2.32	39.6 ± 2.43	12,37±0,49	0,73±0,12	3,12±0,02
C2	0	5.5 ± 0.35 ^{ab}	50.1 ± 6.87 ^{abc}	-20.1 ± 5.44	37.9 ± 2.31	12,67±0,31	1,23±0,03	3,18±0,01
	7	5.9 ± 0.25 ^{a}	53.7 ± 2.23 ^{ab}	-14.4 ± 1.80	38.5 ± 1.71	12,63±0,40	1,09±0,04	3,18±0,01
	14	5.8 ± 0.32 ^{a}	54.5 ± 1.98 ^{ab}	-12.1 ± 2.51	36.9 ± 2.18	12,43±0,15	0,85±0,12	3,24±0,04
	21	6.5 ± 0.85 ^{a}	54.1 ± 2.69 ^{ab}	-14.0 ± 2.32	38.5 ± 2.75	12,5±0,10	0,68±0,05	3,15±0,32
EW1	0	5.5 ± 0.35 ^{ab}	50.1 ± 6.87 ^{abc}	-20.1 ± 5.44	37.9 ± 2.31	12,66±0,31	1,23±0,03	3,18±0,01
	7	5.4 ± 0.25 ^{ab}	53.8 ± 2.25 ^{ab}	-14.4 ± 2.22	38.6 ± 1.84	12,7±0,44	1,06±0,04	3,18±0,02
	14	5.7 ± 0.32 ^{ab}	52.1 ± 4.54 ^{abc}	-10.3 ± 1.92	38.1 ± 2.26	12,6±0,20	0,87±0,14	3,21±0,01
	21	5.6 ± 0.29 ^{ab}	56.2 ± 2.02 ^{ab}	-13.7 ± 2.41	40.2 ± 2.02	12,26±0,51	0,67±0,15	3,18±0,08
EW2	0	5.5 ± 0.35 ^{ab}	50.1 ± 6.87 ^{abc}	-20.1 ± 5.44	37.9 ± 2.31	12,67±0,31	1,23±0,03	3,18±0,01
	7	5.6 ± 0.24 ^{ab}	57.2 ± 2.19 ^{a}	-15.5 ± 2.20	40.1 ± 2.21	12,7±1,06	1,04±0,08	3,17±0,02
	14	5.7 ± 0.31 ^{ab}	54.9 ± 2.92 ^{ab}	-14.2 ± 2.53	38.8 ± 2.28	12,63±0,21	1,02±0,09	3,22±0,01
	21	6.0 ± 0.31 ^{a}	54.9 ± 2.22 ^{ab}	-15.1 ± 3.10	39.5 ± 2.57	12,73±0,21	0,69±0,12	3,17±0,04
EW3	0	5.5 ± 0.35 ^{ab}	50.1 ± 6.87 ^{abc}	-20.1 ± 5.44	37.9 ± 2.31	12,67±0,31	1,23±0,03	3,18±0,01
	7	5.9 ± 0.22 ^{a}	54.1 ±1 .60 ^{ab}	-14.1 ± 1.98	37.7 ± 1.36	13,03±0,15	1,09±0,07	3,16±0,02
	14	6.0 ± 0.35 ^{a}	53.1 ± 3.11 ^{abc}	-12.0 ± 2.42	38.5 ± 2.07	12,23±0,40	0,96±0,11	3,22±0,02
	21	5.6 ± 0.25 ^{ab}	55.3 ± 2.63 ^{ab}	-14.9 ± 1.64	40.3 ± 2.18	12,2±0,35	0,80±0,08	3,16±0,01
EW4	0	5.5 ± 0.35 ^{ab}	50.1 ± 6.87 ^{abc}	-20.1 ± 5.44	37.9 ± 2.31	12,67±0,31	1,23±0,03	3,18±0,01
	7	5.7 ± 0.39 ^{ab}	55.0 ± 1.77 ^{ab}	-14.3 ± 1.26	38.2 ± 1.46	13,3±0,40	1,11±0,06	3,17±0,02
	14	6.1 ± 0.30 ^{a}	51.8 ± 4.45 ^{abc}	-13.5 ± 2.03	38.1 ± 1.89	13,3±0,90	0,99±0,07	3,21±0,04
	21	5.8 ± 0.29 ^{a}	54.9 ± 3.27 ^{ab}	-14.2 ± 2.04	39.6 ± 2.43	12,4±0,52	0,79±0,04	3,19±0,04
Control	0	5.5 ± 0.35 ^{ab}	50.1 ± 6.87 ^{abc}	-20.1 ± 5.44	37.9 ± 2.31	12,67±0,31	1,23±0,03	3,18±0,01
	7	5.7 ± 0.24 ^{a}	54.8 ± 2.31 ^{ab}	-13.0 ± 2.44	35.7 ± 2.11	13,37±0,50	1,04±0,04	3,18±0,02
	14	5.7 ± 0.30 ^{ab}	55.6 ± 2.30 ^{ab}	-13.3 ± 2.69	38.8 ± 2.31	12,93±0,23	1,03±0,07	3,23±0,01
	21	5.1 ± 0.21 ^{b}	48.0 ± 2.49 ^{c}	-4.4 ± 1.17	28.5 ± 3.92	12,5±0,26	0,94±0,15	3,22±0,03

4.3.2. Total phenolic, flavonoids, and flavanol compounds

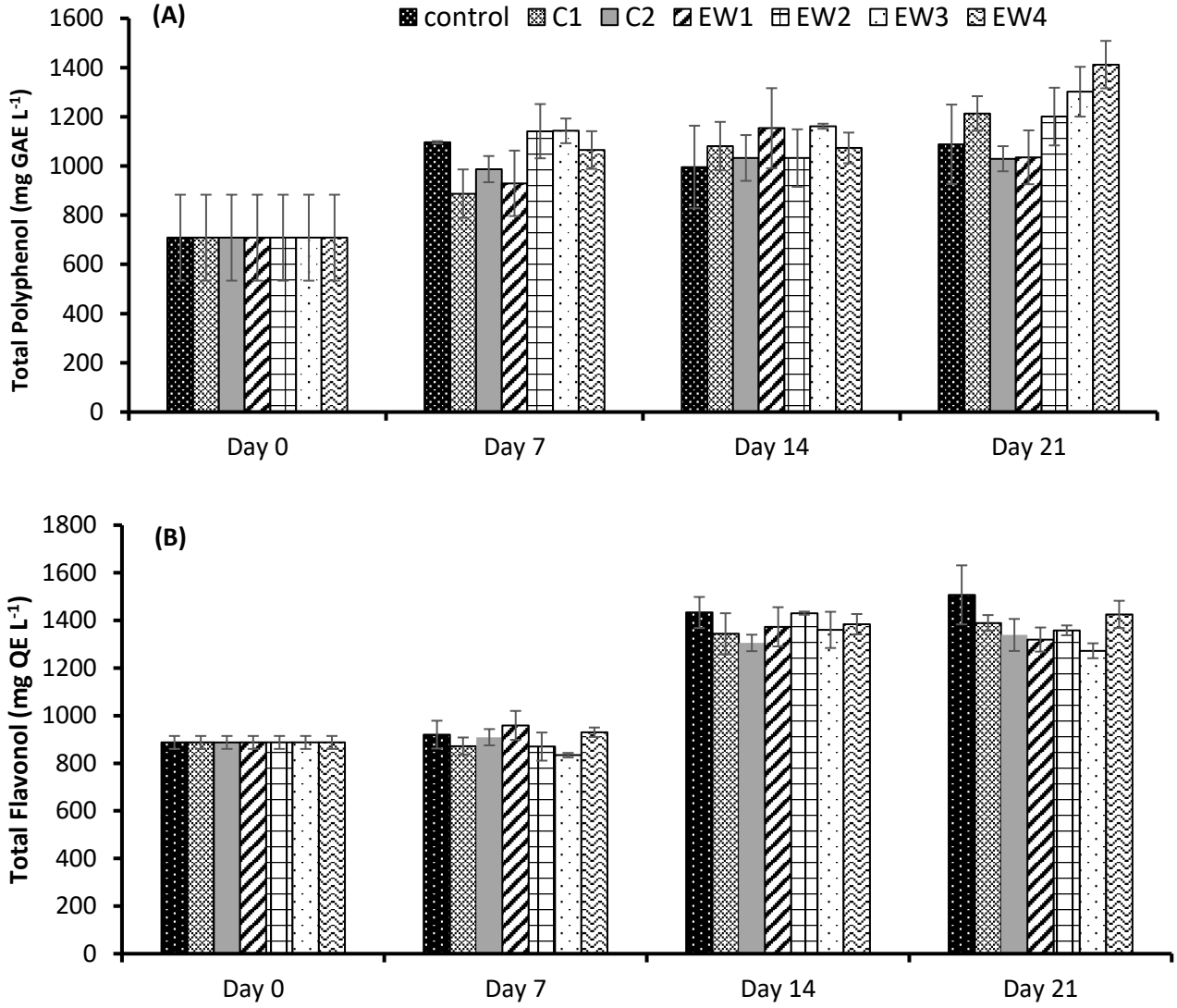
4.3.2.1. Total Phenolic compounds

Storage duration was found to have a significant effect on all treated apples' total phenolic compound ($p \leq 0.05$), as concentration increased continuously across all treatments (Figure. 4.1A). However, treatment of apples alone on a given day did not have a major impact. Phenolics concentration 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 (Figure. 4.1A). At the end of postharvest storage, the highest total phenolic compound (1412 ± 96.4 mg GAE L^{-1}) was found in EW4 treated apples with 200 mg L^{-1} for 15 min. This result agrees with other EW treatment investigations on fresh produce. For example, Li, *et al.* (2020b) demonstrated that AIEW treatment significantly increased the total phenolic concentration of jujube fruit. Compared with chlorinated water-treated apples in this study, the relative influence of AIEW > NaOCl on phenolic compound concentration. Apples are amongst the fruits with the highest content of phenolic compounds. Phenolic compounds are generally recognized as the main determinants of the biological activities in apples with the capability for the prevention of cardiovascular disease, asthma and other lung dysfunctions, diabetes, obesity, and cancer (Boyer and Liu, 2004; Šahinović and Murtić, 2019). Therefore, AIEW treatment has great potential for boosting the synthesis of polyphenols in apple fruit.

4.3.2.2. Total flavonoids and flavanol content

Flavonoids are the main types of pigments of fruit, the content that is related to the appearance of colour of the fruit, and of which, the appearance colour is an important indicator to evaluate the maturity and freshness of fruit (Gao *et al.*, 2019). Flavonols in apples are presented by quercetin glycosides. In this study, immediately after AIEW and chlorinated treatment on days 0 and 7, no significant differences were observed in total flavonol concentration across all treatments and control samples (Figure 4.1C). Overall, the interaction of storage duration and the type of treatment had a significant impact on total flavonol and flavanol concentration ($p \leq 0.05$). The total flavonol content increased from 887 mg QE L^{-1} for all treatment types to ranges of 1272 mg QE L^{-1} to 1507 mg QE L^{-1} at the end of storage. The 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 (Fig. 4.1B). Furthermore, the total flavanol content of apples increased progressively and significantly throughout storage i.e., from 119 ± 25.5 mg CE L^{-1} to 251 ± 29.6 mg CE L^{-1} at the end of storage. On day 21, all samples maintained a high level of flavanol and the dipping treatments did not have a significant impact, besides comparison between C1 (Chlorinated water, 10 min dipping) and control samples.

A similar observation was reported by Li *et al*. (2020b) for jujube fruit treated with slight acidic EW. The authors reported an initial increase in total flavonoids for treated jujube fruit until day 30, and SAEW treatment helped maintain relatively high total flavonoids compared to other treatments at the end of storage. Similar observation was reported by Chen *et al*. (2020), as the authors showed that AEW treatment inhibited the degradation of flavonoids in the pericarp of longan fruit during storage. Maintaining relatively high total flavonoid in fruit up to the end of storage might contribute to reducing decay development and enhancing the resistance to fruit senescence (Zhi *et al.,* 2017). These results demonstrate that SAI-EW treatment could be capable of delaying the degradation of flavonoids as well as boost its production in apples during storage.



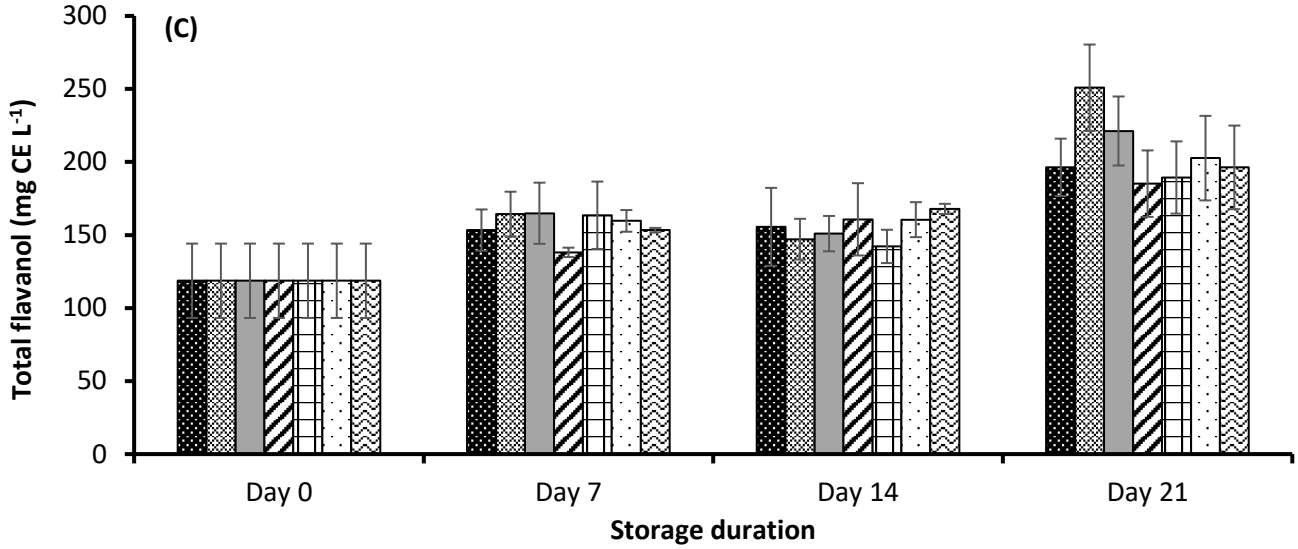


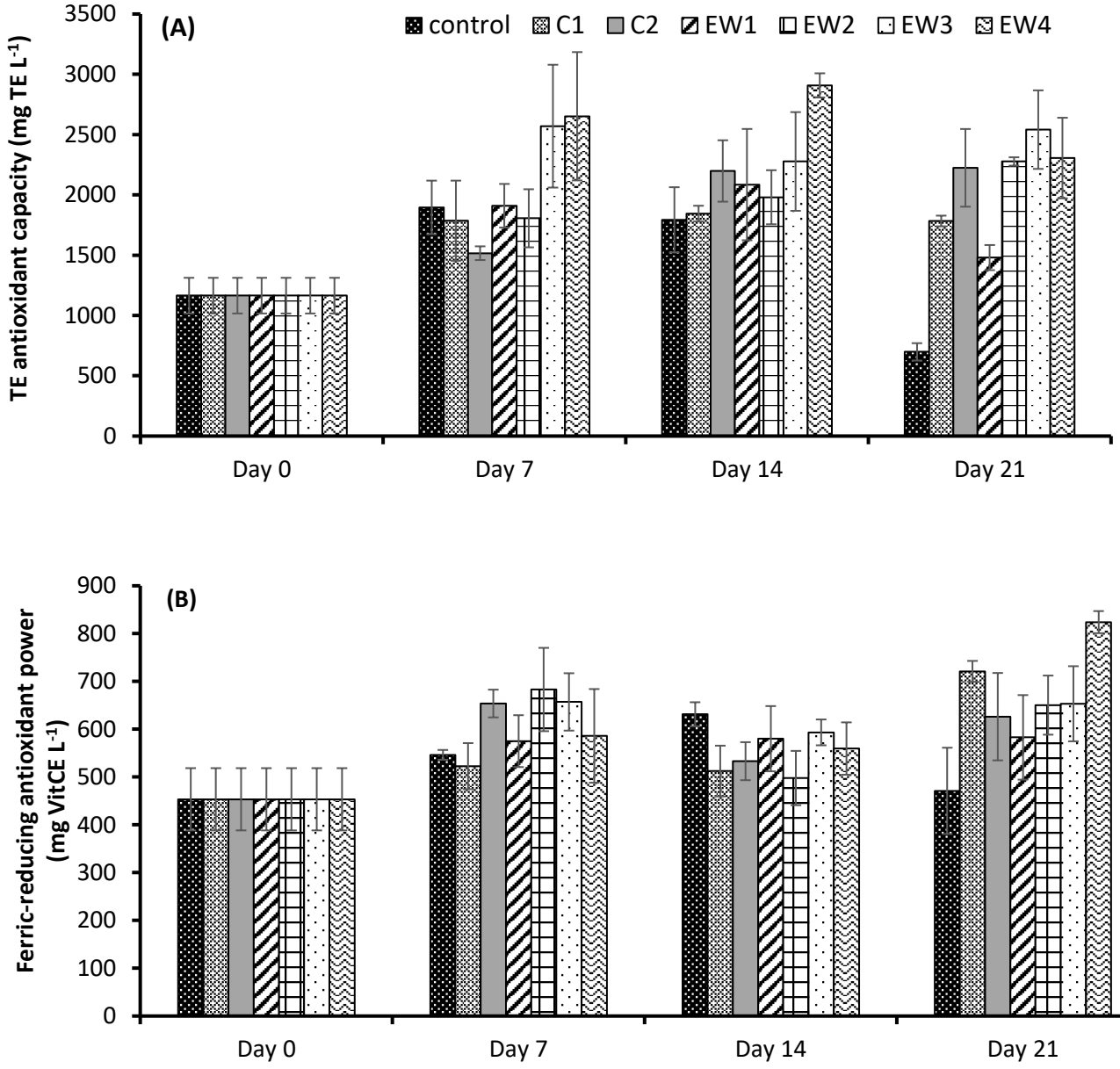
Figure 4.1. Effects of AIEW and chlorinated water treatments on cv. "Granny Smith" apples: polyphenol concentration **(A)**, total flavonol concentration **(B)** and total flavanol concentration **(C)**, during storage at 5 °C and 90 ± 2% RH for 21 days. Error bars represent standard deviation (SD) of mean values (n = 3) tested using Duncan multi-range test at 95% confident interval ($p \leq 0.05$).

4.3.3. Antioxidant capacity

The Trolox equivalent antioxidant capacity (TEAC), ferric-reducing antioxidant capacity (FRAP) and oxygen radical absorbance activity (ORAC) 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 value was observed across all the treatments, besides the control samples that significantly declined after day 14 (Figure. 4.2A). On day 21, the lowest value was observed for control samples (698 ± 72 mg TE L^{-1}), while the highest value was obtained in EW3-treated apples (AIEW 200 mg L^{-1} for 10 min). However, the EW3-treated apples were not significantly different from C2, EW2, and EW4 (Figure. 4.2A). Similarly, a significant and continuous increase in FRAP value was observed across all the treatments as the storage progressed, while control samples declined after day 14 ($p < 0.05$). At the end of storage (day 21), the highest and lowest values were found in EW4-treated (824 ± 23.1 mg VitCE L^{-1}) and control samples (471 ± 90 mg VitCE L^{-1}), respectively (Figure. 4.2B).

In contrast, the ORAC value fluctuated for all treated and non-treated apples during storage (Figure. 4.2C). In comparison to day 0 samples, a significant increase in oxygen radical scavenging activity was observed on day 7 for all treated apples and controls, with exception of C1, C2, and EW1 apples ($p \leq 0.05$). The highest ORAC values were noted in control, EW3 and EW4 samples on day 7; in C1, C2, and EW1-treated apples on day 14 were and only C1 samples (5074 ± 210.1 mg TE L^{-1}) on day 21 (Figure. 4.2C). Other studies on the application of EW treatments on fresh fruit are in agreement with the findings from this study. They demonstrated the various EW treatments better maintained or elevated levels of

antioxidant capacity compared to control samples (Tang *et al*., 2021; Li *et al.,* 2020b). Radical scavenging ability and reducing power are also important indicators of the antioxidant capacity of plants (Chen *et al.* 2019b). Overall, compared to other treatments and the control, EW treatment adequately maintained a relatively high TEAC, FRAP and ORAC values or could elevate the antioxidant activities of 'Granny Smith' apples.



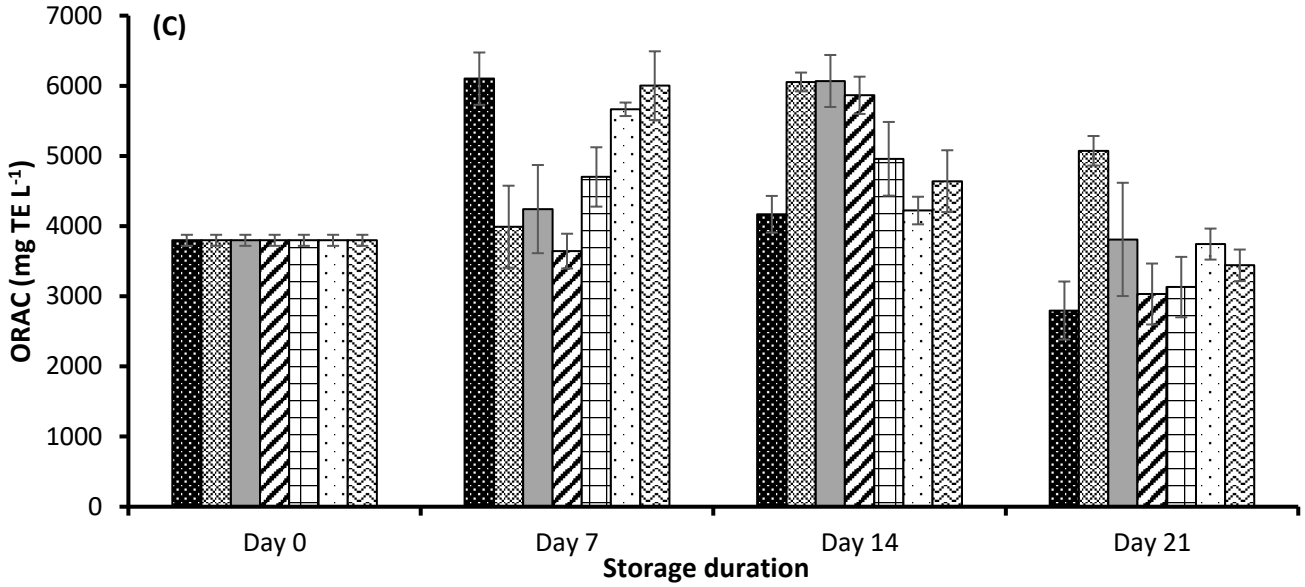


Figure 4.2. Effects of AIEW treatments and chlorinated water treatments on cv. "Granny Smith" apples: Trolox equivalent antioxidant capacity **(A)**, total Ferric-reducing antioxidant power **(B)**, and total oxygen radical absorbance capacity **(C)** during storage at 5 °C and 90 ± 2% RH for 21 days. Error bars represent standard deviation (SD) of mean values (n = 3) tested using Duncan multi-range test at 95% confident interval ($p \leq 0.05$).

4.3.4 Physiological disorder

4.3.4.1. Superficial scald incidence (%)

Superficial scald is a physiological disorder that occurs during cold storage (below 10 -15°C) of some important apple cultivars such as cv. "Granny Smith" and "Red Delicious" (Singh *et al.*, 2019). It is characterized by brown discoloration of irregularly shaped areas on the surface of the fruit during or following storage (Ramokonyane *et al.*, 2016). Development of superficial scald on 'Granny Smith' apple was evaluated at day 0, 7, 14 and 21 after treatments with electrolysed water and sodium hypochlorite. After treatment, the scald incidence was recorded as a percentage of the fruit affected by the scald (Table 4.3). There was no superficial scald on the apple fruit on day 0 for all treatment types (Table 4.3). The severity of superficial scald increased with days of shelf life on 'Granny Smith' apples in this study. At the end of storage duration, fruit under EW4 (AIEW 200 mg L^{-1} for 15 min), C2 and C1 (Chlorinated water, for 10 min dipping) had superficial scald 76-100 % and fruit treated with electrolysed water EW2 (AIEW 300 mg L^{-1} for 15 min) and EW3 (AIEW 300 mg L^{-1} for 10 min) had the lowest scald severity. These results are in agreement with the study reported by Rico *et al.* (2008) for the use of neutral electrolysed water (EW) for quality maintenance and shelf-life extension of minimally processed lettuce and Marc *et al.*, (2020) for the post-harvest acclimation to cold prevent from superficial scald development in 'Granny Smith' apples.

These studies demonstrated that severity of superficial scald increased with the storage duration.

Early research determined that scald could be prevented by storing apples with substances that can absorb volatile compounds (oiled and wax wraps, charcoal, sawdust, etc.) (Scott *et al.*, 1995; Ju and Curry, 2000). Additionally, a variety of physical stressors can be imposed prior to cold storage to accomplish either localized or whole-fruit scald prevention (Gapper *et al.*, 2017). Post-harvest management strategies have been limited to the use of controlled atmosphere (CA) storage (Vuković *et al*., 2020) and postharvest treatments, such as the antioxidant, diphenylamine (DPA) (Jung and Watkins, 2008; Lurie and Watkins, 2012; Karagiannis *et al*., 2018) and 1-methylcyclopropene (1-MCP) (Jung and Watkins, 2008; Poirier et al., 2020). In the current study, the apple fruit were not subjected to any treatments after harvest and were stored for 3 months in cold storage before treatment with SAI-EW and sodium hypochlorite. In a similar study, Vuković *et al*. (2020) reported the effect of red photo selective nets on 'Granny Smith' apples against superficial scald. The fruit were stored in regular air storage at 0°C for 4 months and then kept for 7 days at room temperature (shelf life). The authors found that after cold storage, superficial scald severity was significantly higher in fruits under red net. The authors concluded that it is important to apply prevention measures such as 1-MCP and CA storage on superficial scald-susceptible apple varieties. Diphenylamine (DPA) or 1-methylcyclopropene (1-MCP) treatment can be used to control scald, but application is required within the first weeks of cold air storage following harvest (Tomic *et al.*, 2016; Poirier *et al.*, 2020). After this period, treatments are no longer effective due to irreversible physiological changes, hereby referred to as "scald induction" (Tomic *et al.*, 2016). As a recent report indicates, apple flesh softening can be reduced by 1-MCP treatment following ultra-low oxygen CA (ULO-CA) storage ($pO_2 \leq 1.0$ kPa) (Poirier *et al.*, 2020). Poirier et al. (2020), investigated the effects of ultra-low oxygen CA (ULO-CA) against superficial scald on 'Granny Smith' apples treated with hot water (48 °C for 3 min) and 1-MCP at harvest or following long-term (>3 months) in-house. Different CA storage environments (2.0 kPa and 1.0 kPa O_2) and delays in ULO-CA establishment were used in combinations with delayed DPA and 1-MCP treatments to reveal the scald induction timeline and how different storage practices may impact the effectiveness of post-CA scald control measures. Taken together, the results indicated that scald induction was effectively delayed during ULO-CA storage and resumed upon return to air storage. 1-MCP and hot water treatments applied after four months of ULO-CA storage were equally effective at controlling scald during subsequent air storage as treatments applied at harvest (Poirier *et al.*, 2020). However, the efficacy of post-storage treatments relied on the rapid establishment and maintenance of ULO conditions and immediate treatment upon removal from ULO-CA storage. In a recent study by Kawhena *et al.* (2021) the efficacy of dynamic controlled atmosphere technologies, i.e., repeated low

oxygen stress (RLOS) and dynamic controlled atmosphere-chlorophyll fluorescence (DCA-CF) to control superficial scald development on ‘Granny Smith’ apples during long-term storage was studied. The authors found that the development of superficial scald was inhibited for up to 10 months and 7 d shelf life (20 °C) under RLOS + ULO and DCA-CF treatments. Therefore, the efficacy in controlling scald severity of ‘Granny Smith’ apples treated with electrolysed water and sodium hypochlorite was not as effective. It is suggested that the fruit be treated with 1-MCP before treatments to achieve a high efficacy in controlling scald severity. Further investigations in the effect of EW and 1-MCP on apples are recommended in order to explore this alternative that might reduce the severity of superficial scald.

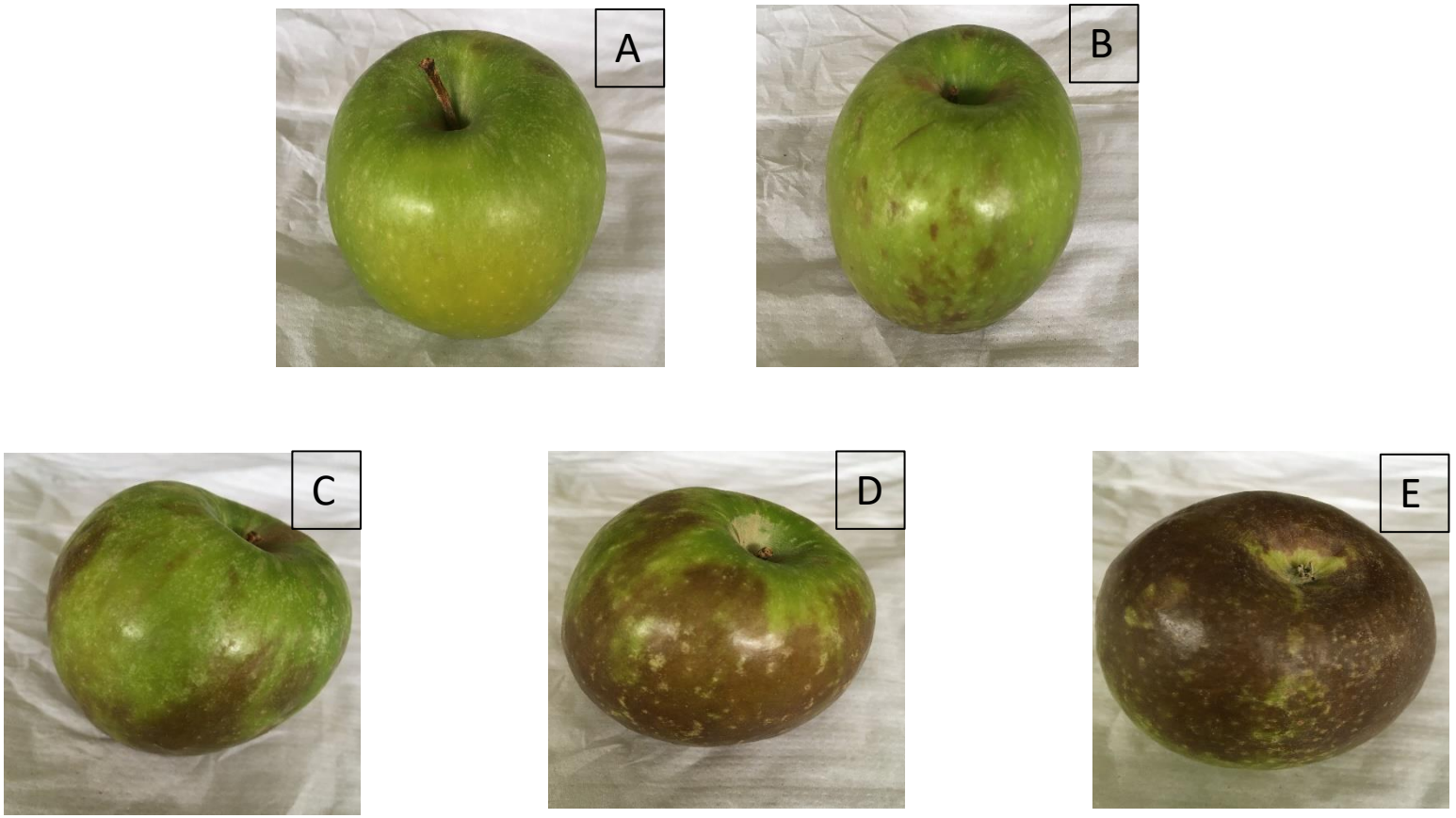


Figure 4.3. Grading of superficial scald incidence on 'Granny Smith' apples A = Grade 0 (no scald), B = Grade 1 (light scald 1-25%), C = Grade 2 (moderate scald 26-50%), D = Grade 3 (severe scald 50-75%) and E = Grade 4 (extreme scald 76-100%).

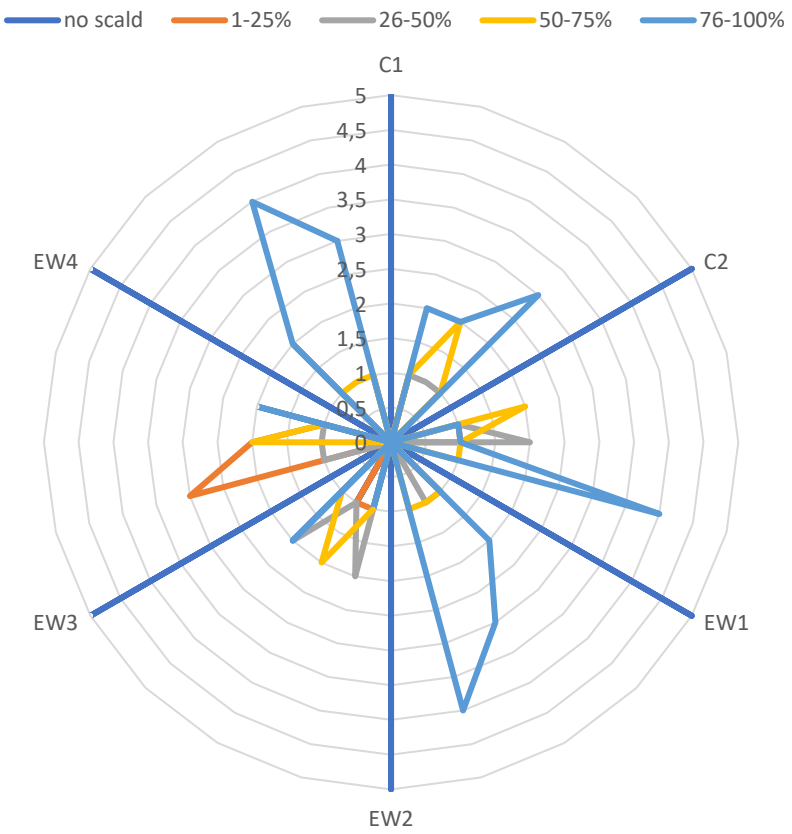


Figure 4.4. Effect of different treatments on superficial scald of ‘Granny Smith’ apple stored at 5 °C and 90 ± 2% RH for 21 days.

4.3.5. Microbial analysis

Results obtained showed that all the treatments exhibited significant decontaminating activity against naturally occurring microbial population on the surface of the apple fruit (Figure 4.4). Compared to the non-treated control samples, ≈ 2 Log reduction in total aerobic mesophilic bacteria (from 4.1 Log CFU cm^2 to 2.2 Log CFU cm^2) and < 1 Log reduction for yeast and mould (from 3.9 Log CFU cm^2 to 2.7 Log CFU cm^2) count was recorded on day 7. Samples treated under EW4 showed the greatest reduction for total aerobic mesophilic bacteria compared to other treatments on day 7 and remained significantly lower than the control sample at the end of storage (Figure 4.4). A comparison of relative influence for decontamination of fruit surface showed that EW4 > EW2 = C1 > control at the end of storage for total aerobic mesophilic bacteria. In contrast, for the yeast and mould count, the AIEW treatments were most effective compared to conventional chlorine treatment. These findings are in agreement with other studies on the comparative efficacy of EW treatments to chlorinated water. Koide *et al*. (2009) revealed that slightly acidic electrolyzed water had an equivalent or high disinfectant efficacy for fresh-cut cabbage compared to NaOCl solution. The authors showed that EW treatment reduced total aerobic bacteria by ≈ 1.5 Log CFU g^{-1} and yeasts and moulds by 1.3 Log CFU g^{-1} on fresh-cut cabbage. Similarly, Ding *et al*. (2015) found that EW treatment reduced total aerobic bacteria and yeasts and moulds by ≈ 1.45 Log CFU g^{-1} and ≈ 1.10 Log CFU g^{-1}, respectively on 'Cherry' tomatoes, and 0.93 Log CFU g^{-1} and 0.96 Log CFU g^{-1}, respectively, for strawberries. Ogunniyi *et al*. (2021) found that bactericidal efficacy of slightly acidic electrolyzed water showed bactericidal effect against natural microbial load and reduced survival population of *E. coli* and *L. innocua* on fresh spinach leaves.

Overall, the different dipping duration in AIEW at a given concentration had no significant impact ($p > 0.05$) on the efficacy of the treatment to reduce microbial load. This suggests that 10 min dipping in AIEW would be sufficient for the treatment of 'Granny Smith' apples within the pack-house. Most importantly, the EW treatment has shown a strong decontamination effect compared to the conventional industry practice. The antimicrobial activity of EW treatment could be attributed to various factors; the presence of free reactive chlorine species, the low pH (in the case of acidic electrolyte water), and other studies have suggested that this activity is due to its high ORP (Kim *et al.*, 2000; Park *et al*., 2004; Colangelo *et al.,* 2015). The ORP of EW, which represents the strength of ions to accept/donate electrons (Reduction/Oxidation), has been suggested as a strong decontaminating factor relative to free chlorine (Kim *et al*., 2000). However, further studies on the mechanism of microbial decontamination/mode of action on the fruit surface, and the impact of long-term cold storage

on EW efficacy as well as the combined effects with other postharvest technologies is required.

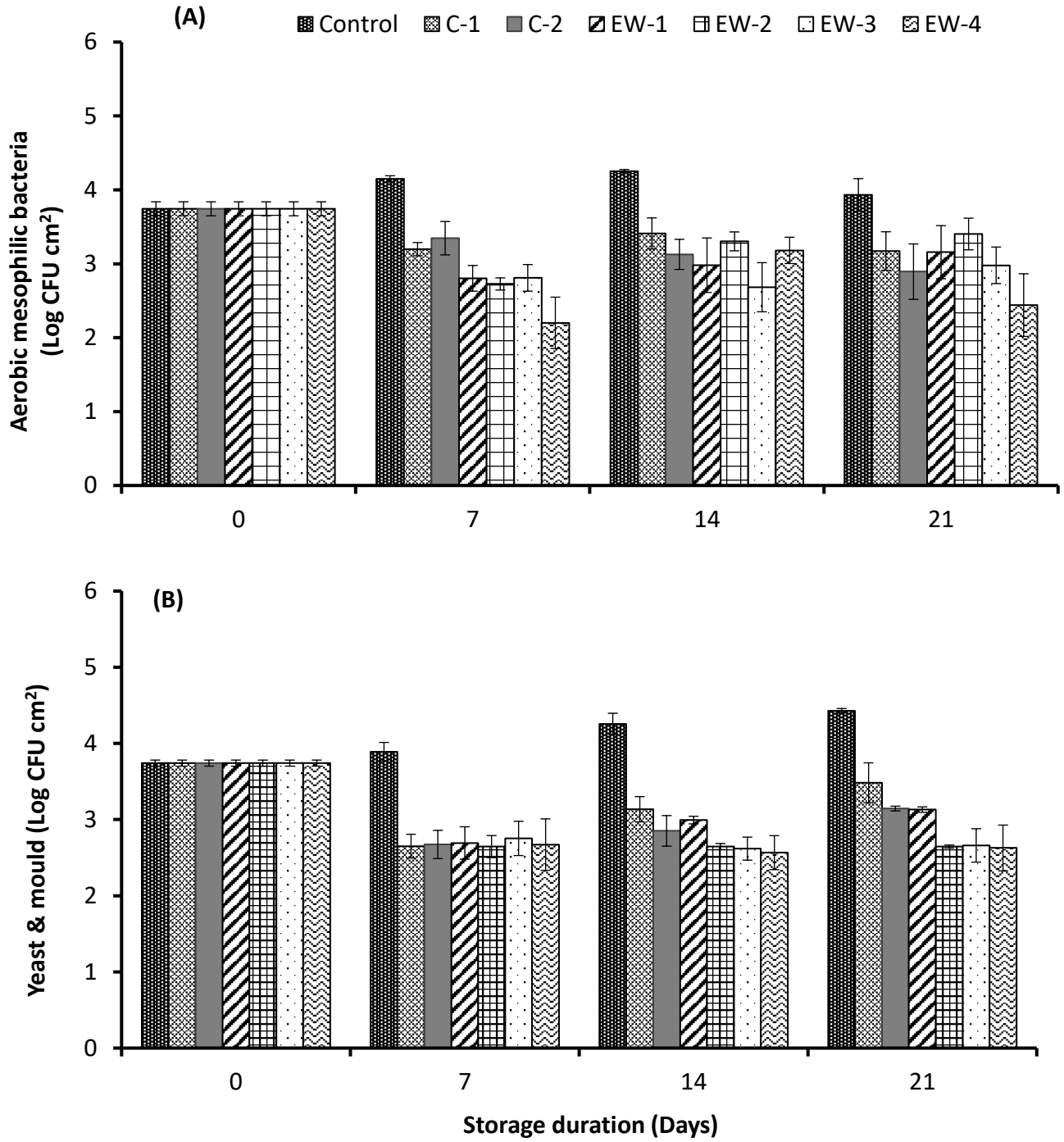


Figure 4.5. Effects of AIEW treatments and chlorinated water treatments on the 'Granny Smith' apple surface microbial load: **(A)** total aerobic mesophilic bacteria and **(B)** yeast and moulds, during storage at 5 °C and 90 ± 2% RH for 21 days. Error bars represent standard

deviation (SD) of mean values (n = 3) tested using Duncan multi-range test at 95% confidence interval ($p \leq 0.05$).

4.4. Conclusion

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 AIEW 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. Additionally, compared to the control this application was effective in enhancing and maintaining total polyphenol, flavonol, flavanol, and antioxidant capacity of 'Granny Smith' apples. Compared with the control and chlorinated water treatment, the AIEW treatment ensured a high reduction in natural microbial load. Therefore, AIEW dipping can be considered as an effective alternative to chlorine wash pre-treatment to control natural spoilage microorganisms in the fresh apple pack-house. Additionally, in order to minimize superficial scald incidence prevention measures such as 1-MCP should be applied.

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Supplementary Files

Table S1. Pearson correlation matrix between various measured quality parameters during storage of 'Granny Smith' apples.

Variables	Texture	L*	a*	b*	*h°*	*C**	TSS	TA	TSS_TA	pH	TEAC	Flavanol	Flavonol	TP	AMB	Yeasts & Moulds
Texture	**1**	**0,474**	-0,261	**0,565**	0,040	0,133	0,182	-0,298	0,280	0,129	**0,583**	**0,419**	0,245	**0,497**	**-0,439**	**-0,640**
L*	**0,474**	**1**	**-0,536**	**0,903**	**0,399**	0,302	-0,013	**-0,537**	**0,505**	-0,198	**0,578**	**0,630**	0,227	**0,643**	**-0,493**	**-0,567**
a*	-0,261	**-0,536**	**1**	-0,313	-0,231	**-0,552**	-0,136	-0,191	0,169	**0,390**	-0,235	-0,333	**0,487**	-0,008	0,243	**0,377**
b*	**0,565**	**0,903**	-0,313	**1**	**0,539**	0,298	-0,085	**-0,702**	**0,649**	-0,179	**0,692**	**0,698**	**0,440**	**0,740**	**-0,610**	**-0,698**
h°	0,040	**0,399**	-0,231	**0,539**	**1**	**0,508**	-0,010	-0,329	0,303	-0,228	0,291	0,373	0,183	0,229	-0,237	-0,328
C*	0,133	0,302	**-0,552**	0,298	**0,508**	**1**	-0,358	-0,131	0,123	**-0,413**	0,026	**0,483**	-0,006	0,011	-0,204	-0,361
TSS	0,182	-0,013	-0,136	-0,085	-0,010	-0,358	**1**	**0,390**	-0,320	0,074	0,125	-0,298	-0,324	-0,185	0,271	0,121
TA	-0,298	**-0,537**	-0,191	**-0,702**	-0,329	-0,131	**0,390**	**1**	**-0,986**	0,146	**-0,618**	**-0,754**	**-0,790**	**-0,804**	**0,433**	0,341
TSS_TA	0,280	**0,505**	0,169	**0,649**	0,303	0,123	-0,320	**-0,986**	**1**	-0,199	**0,627**	**0,762**	**0,752**	**0,770**	**-0,387**	-0,289
pH	0,129	-0,198	**0,390**	-0,179	-0,228	**-0,413**	0,074	0,146	-0,199	**1**	-0,155	**-0,518**	0,311	-0,098	0,242	0,157
TEAC	**0,583**	**0,578**	-0,235	**0,692**	0,291	0,026	0,125	**-0,618**	**0,627**	-0,155	**1**	**0,686**	0,348	**0,711**	**-0,553**	**-0,637**
Flavanol	**0,419**	**0,630**	-0,333	**0,698**	0,373	**0,483**	-0,298	**-0,754**	**0,762**	**-0,518**	**0,686**	**1**	**0,416**	**0,765**	**-0,558**	**-0,487**
Flavonol	0,245	0,227	**0,487**	**0,440**	0,183	-0,006	-0,324	**-0,790**	**0,752**	0,311	0,348	**0,416**	**1**	**0,612**	-0,123	-0,090
TP	**0,497**	**0,643**	-0,008	**0,740**	0,229	0,011	-0,185	**-0,804**	**0,770**	-0,098	**0,711**	**0,765**	**0,612**	**1**	**-0,610**	**-0,488**
AMB	**-0,439**	**-0,493**	0,243	**-0,610**	-0,237	-0,204	0,271	**0,433**	**-0,387**	0,242	**-0,553**	**-0,558**	-0,123	**-0,610**	**1**	**0,824**
Yeasts & Moulds	**-0,640**	**-0,567**	**0,377**	**-0,698**	-0,328	-0,361	0,121	0,341	-0,289	0,157	**-0,637**	**-0,487**	-0,090	**-0,488**	**0,824**	**1**

Values in bold are different from 0 with a significance level alpha = 0.05. Trolox equivalent antioxidant capacity - TEAC; Total Polyphenols – TP; Aerobic Mesophilic Bacteria - AMB

Table S2. The *p*-values of correlated variables measured during storage of 'Granny Smith' apples.

Variables	Texture	L*	a*	b*	*h°*	*C**	TSS	TA	TSS_TA	pH	TEAC	Flavanol	Flavonol	TP	AMB	Yeasts & Moulds
Texture	**0**	**0,011**	0,179	**0,002**	0,840	0,499	0,354	0,124	0,149	0,511	**0,001**	**0,027**	0,210	**0,007**	**0,019**	**0,000**
L*	**0,011**	**0**	**0,003**	**< 0.0001**	**0,035**	0,119	0,946	**0,003**	**0,006**	0,313	**0,001**	**0,000**	0,244	**0,000**	**0,008**	**0,002**
a*	0,179	**0,003**	**0**	0,104	0,236	**0,002**	0,491	0,329	0,391	**0,040**	0,229	0,083	**0,009**	0,969	0,212	**0,048**
b*	**0,002**	**< 0.0001**	0,104	**0**	**0,003**	0,124	0,665	**< 0.0001**	**0,000**	0,362	**< 0.0001**	**< 0.0001**	**0,019**	**< 0.0001**	**0,001**	**< 0.0001**
h°	0,840	**0,035**	0,236	**0,003**	**0**	**0,006**	0,959	0,088	0,117	0,243	0,132	0,050	0,352	0,242	0,225	0,088
C*	0,499	0,119	**0,002**	0,124	**0,006**	**0**	0,061	0,507	0,534	**0,029**	0,897	**0,009**	0,976	0,957	0,298	0,059
TSS	0,354	0,946	0,491	0,665	0,959	0,061	**0**	**0,040**	0,097	0,708	0,525	0,124	0,093	0,346	0,164	0,539
TA	0,124	**0,003**	0,329	**< 0.0001**	0,088	0,507	**0,040**	**0**	**< 0.0001**	0,457	**0,000**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0,021**	0,076
TSS_TA	0,149	**0,006**	0,391	**0,000**	0,117	0,534	0,097	**< 0.0001**	**0**	0,309	**0,000**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0,042**	0,136
pH	0,511	0,313	**0,040**	0,362	0,243	**0,029**	0,708	0,457	0,309	**0**	0,432	**0,005**	0,108	0,618	0,214	0,426
TEAC	**0,001**	**0,001**	0,229	**< 0.0001**	0,132	0,897	0,525	**0,000**	**0,000**	0,432	**0**	**< 0.0001**	0,069	**< 0.0001**	**0,002**	**0,000**
Flavanol	**0,027**	**0,000**	0,083	**< 0.0001**	0,050	**0,009**	0,124	**< 0.0001**	**< 0.0001**	**0,005**	**< 0.0001**	**0**	**0,028**	**< 0.0001**	**0,002**	**0,009**
Flavonol	0,210	0,244	**0,009**	**0,019**	0,352	0,976	0,093	**< 0.0001**	**< 0.0001**	0,108	0,069	**0,028**	**0**	**0,001**	0,534	0,650
TP	**0,007**	**0,000**	0,969	**< 0.0001**	0,242	0,957	0,346	**< 0.0001**	**< 0.0001**	0,618	**< 0.0001**	**< 0.0001**	**0,001**	**0**	**0,001**	**0,008**
AMB	**0,019**	**0,008**	0,212	**0,001**	0,225	0,298	0,164	**0,021**	**0,042**	0,214	**0,002**	**0,002**	0,534	**0,001**	**0**	**< 0.0001**
Yeasts & Moulds	**0,000**	**0,002**	**0,048**	**< 0.0001**	0,088	0,059	0,539	0,076	0,136	0,426	**0,000**	**0,009**	0,650	**0,008**	**< 0.0001**	**0**

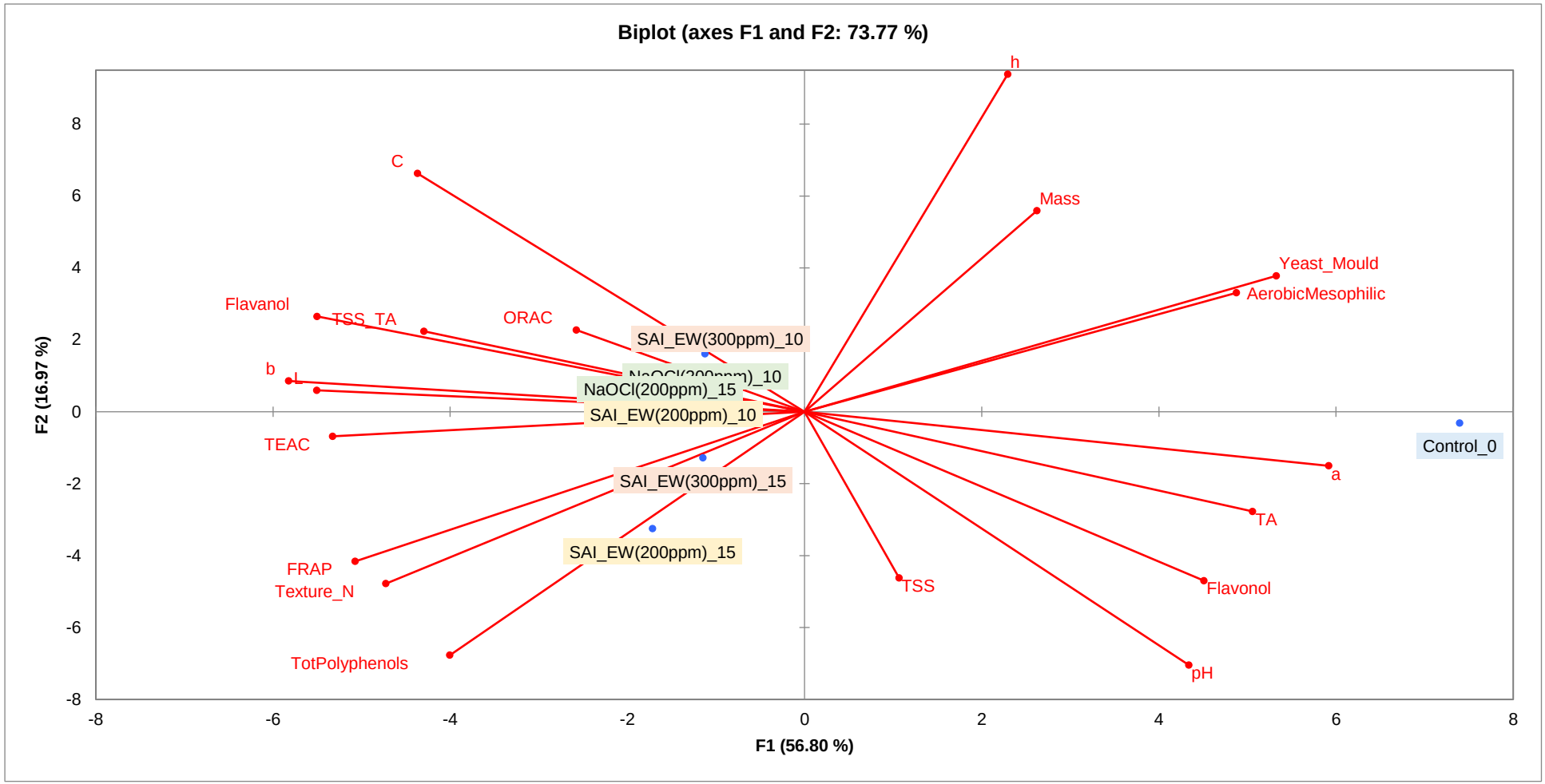
Values in bold are different from 0 with a significance level alpha = 0.05. Trolox equivalent antioxidant capacity - TEAC; Total Polyphenols – TP; Aerobic Mesophilic Bacteria - AMB

Table S3. Coefficients of determination (R^2) values of variables during PCA correlation analysis

Variables	Texture	L*	a*	b*	*h°*	*C**	TSS	TA	TSS_TA	pH	TEAC	Flavanol	Flavonol	TP	AMB	Yeasts & Moulds
Texture	**1**	0,224	0,068	0,319	0,002	0,018	0,033	0,089	0,078	0,017	0,340	0,175	0,060	0,247	0,193	0,410
L*	0,224	**1**	0,287	0,815	0,159	0,091	0,000	0,289	0,255	0,039	0,334	0,397	0,052	0,414	0,243	0,322
a*	0,068	0,287	**1**	0,098	0,053	0,304	0,018	0,037	0,028	0,152	0,055	0,111	0,238	0,000	0,059	0,142
b*	0,319	0,815	0,098	**1**	0,291	0,089	0,007	0,492	0,421	0,032	0,478	0,487	0,194	0,547	0,372	0,487
h°	0,002	0,159	0,053	0,291	**1**	0,259	0,000	0,108	0,092	0,052	0,085	0,139	0,033	0,052	0,056	0,108
C*	0,018	0,091	0,304	0,089	0,259	**1**	0,128	0,017	0,015	0,171	0,001	0,233	0,000	0,000	0,042	0,131
TSS	0,033	0,000	0,018	0,007	0,000	0,128	**1**	0,152	0,102	0,006	0,016	0,089	0,105	0,034	0,073	0,015
TA	0,089	0,289	0,037	0,492	0,108	0,017	0,152	**1**	0,972	0,021	0,382	0,568	0,623	0,646	0,187	0,116
TSS_TA	0,078	0,255	0,028	0,421	0,092	0,015	0,102	0,972	**1**	0,040	0,393	0,581	0,566	0,594	0,150	0,084
pH	0,017	0,039	0,152	0,032	0,052	0,171	0,006	0,021	0,040	**1**	0,024	0,268	0,097	0,010	0,059	0,025
FRAP	0,229	0,496	0,110	0,541	0,075	0,056	0,040	0,405	0,372	0,112	0,393	0,702	0,097	0,788	0,479	0,311
ORAC	0,144	0,080	0,001	0,071	0,000	0,049	0,235	0,000	0,000	0,017	0,154	0,006	0,003	0,042	0,033	0,065
TEAC	0,340	0,334	0,055	0,478	0,085	0,001	0,016	0,382	0,393	0,024	**1**	0,471	0,121	0,505	0,306	0,406
Flavanol	0,175	0,397	0,111	0,487	0,139	0,233	0,089	0,568	0,581	0,268	0,471	**1**	0,173	0,586	0,312	0,237
Flavonol	0,060	0,052	0,238	0,194	0,033	0,000	0,105	0,623	0,566	0,097	0,121	0,173	**1**	0,374	0,015	0,008
TP	0,247	0,414	0,000	0,547	0,052	0,000	0,034	0,646	0,594	0,010	0,505	0,586	0,374	**1**	0,372	0,238
AMB	0,193	0,243	0,059	0,372	0,056	0,042	0,073	0,187	0,150	0,059	0,306	0,312	0,015	0,372	**1**	0,680
Yeasts & Moulds	0,410	0,322	0,142	0,487	0,108	0,131	0,015	0,116	0,084	0,025	0,406	0,237	0,008	0,238	0,680	**1**

Values in bold are different from 0 with a significance level alpha = 0.05. Trolox equivalent antioxidant capacity - TEAC; Total Polyphenols – TP; Aerobic Mesophilic Bacteria - AMB

Supplementary Figure



CHAPTER 5

Characterisation of microbial dynamics on the surface of 'Granny Smith' apples treated with electrolysed water during cold storage

Abstract

Chemical washing/dipping step is a major decontamination practice along the apple fruit value chains to minimize decay. Electrolysed water (EW) treatment as an emerging alternative to chlorinated water has been proven to be effective in reducing microbial load. However, the microbiome response to EW treatment is still unknown. Thus, this study aimed at: (i) characterizing the microbial community isolated from the surface of 'Granny Smith' apples treated with electrolysed water (AIEW) in comparison to chlorinated water and non-treated samples, and (ii) evaluating the efficacy of the treatments on microbial dynamics. Results obtained showed that pre-treatments prior to storage at low temperature and storage duration had a strong impact on the microbial diversity and population dynamics. Electrolysed water treatment resulted in approximately 2 Log reduction in microbial load on the apple surface. Based on morphological identification about 87 pure colonies (56 bacteria and 31 fungi) were tentatively isolated across all treated samples from day 0 to 21. Out of the total 56 identified bacteria only 27 out of these isolates resulted in genetically diverse bacteria population via the enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR). Based on the 16S rDNA sequencing of the dominant bacteria community, a total of eight different bacteria species were identified on the surface of treated and non-treated apples. In addition, based on random amplification of polymorphic DNA (RAPD-PCR) using primers 1283, the 31 fungal isolates were tentatively linked to ten fungal distinct clusters. However, only five out of the ten fungal isolates could be amplified via their ITS1 and ITS4 primers within the 100-1500 bp region. Our results provide first insights into the microbiome response on the surface of 'Granny Smith' apples to EW treatment.

Keywords: Microbial community, population dynamics, post-harvest management, decontamination, intergenic spacer (ITS) region, *Malus domestica Borkh*.

5.1. Introduction

Post-harvest diseases destroy 10–30% of the total yield of fruit and vegetable crops during handling, transportation, storage and marketing. Therefore, preventing the proliferation and development of post-harvest microbial spoilage and pathogens during storage is an important challenge for maintaining apple fruit quality and safety. Domesticated apple (*Malus* × *domestica* Borkh) belongs to the Rosaceae family, and Pyreae tribe. Currently, it is one of the major temperate fruit crops grown across northern and southern latitudes and consumed globally. Apple is a rich source of phytonutrients such as dietary fibre, polyphenols, flavonoids and antioxidant activity (Lee *et al.*, 2003; Duda-Chodak *et al.*, 2011; Büchner, 2015; Wang *et al.*, 2015; Musacchi and Serra, 2018). Apple fruit surface is colonized by a number of different microorganisms during post-harvest, however, during long-term storage it is susceptible to spoilage and infections by several fungal pathogens (Abdelfattah *et al.,* 2016; Abdelfattah *et al*., 2021).

Chlorine-based solution prepared commercially from sodium hypochlorite (NaOCl) with concentrations, ranging from 5 mg L^{-1} - 200 mg L^{-1} is the most used sanitizing agent for washing fresh produce including apples and has been authorized for use by the South African Foodstuffs, Cosmetics and Disinfectants Act. At effective concentration treatment with NaOCl results in a reduction of pathogens on fresh produce (Beltrán *et al.*, 2005; Issa-Zacharia *et al.*, 2011; Li *et al.*, 2020). At higher concentrations for increased effectiveness may cause product tainting, and result in the deposition of chemical residues on the product (Li *et al.*, 2020). The risks of chemical residues on fresh ready to eat product have raised public health concerns regarding the use of chlorinated water treatments, leading to additional regulatory barriers, limitations, safety, and regulations for its use (Ayebah and Hung, 2005; Tucker and Featherstone, 2010; Nan *et al.*, 2019). In addition, increasing consumer demand for safe, and fresh produce, has heightened the need for effective, environmentally friendly and low-cost preservation alternatives for apple fruit.

Electrolyzed water (EW) has excellent antimicrobial properties against several microbial pathogens and it is gaining popularity in the food industry (Hao *et al.*, 2011; Colangelo *et al.*, 2015; Yang *et al.*, 2020; Youssef and Hussien, 2020). It is produced through the electrolysis of salt solution in a chamber with an anode and a cathode separated by a diaphragm membrane (Hayta and Aday, 2015; Rahman *et al.*, 2016; Iram *et al.*, 2021). The most common salt used in this process is sodium chloride (NaCl), but a mixture of potassium chloride (KCl) and Magnesium chloride ($MgCl_2$) have also been reported (Colangelo *et al.*, 2015; Youssef and Hussien, 2020). Through the electrolysis process, two different characteristics streams of active water are produced: (a) an alkaline water (pH 11-13, oxidation-reduction potential (ORP), and −795 to -900 mV) produced from the cathode side (Iram *et al.*, 2021), hereinafter called alkaline electrolyzed water (AIEW); and (b) a strong acid

solution produced from the anode side (high ORP from >1000 mV, and pH 2-3), hereinafter called acidic electrolyzed water (AEW)(Huang *et al.*, 2006; Huang *et al.*, 2008; Rahman, Khan, & Oh, 2016). On the other hand, some systems do not have a diaphragm membrane, and neutral electrolysed water at pH: 7-8, ORP: 750 mV is generated, as the HCl formed at the anode side neutralizes the NaOH at cathode side (Guentzel *et al.*, 2011; Han *et al.*, 2018). Washing with EW is a promising alternative to chlorinated water to inhibit food-borne pathogens.

However, even though these developments are promising, there are still missing links between post-harvest diseases on apples and their colonizing microbiota following post-harvest treatments with EW treatments. According to Wassermann *et al.* (2019), the apple fruit surface is teeming with a wide variety of spoilage or pathogenic microorganisms, which are closely associated with fruit post-harvest deterioration. Recent studies have shown that different apple fruit harbour distinctly different fungal and bacterial communities that vary in diversity and abundance (Abdelfattah *et al.*, 2016; Liu *et al.*, 2018; Wassermann *et al.*, 2019b; Abdelfattah *et al.*, 2021a; Kusstatscher *et al.*, 2021). Post-harvest practices along with cold storage could influence the natural microbiota on the apples, which could eliminate beneficial populations. Therefore, further research elucidating the major microbial community and population dynamics on apple fruit as a function of postharvest treatments and storage duration is crucial.

Many techniques have been developed for the identification of microorganisms. This includes the use of conventional morphology and molecular methods. Molecular tools allow for rapid and accurate identification of microbial populations. Polymerase chain reaction (PCR), is a molecular based technique that provides researchers with a simple, economic and fast approach for microbial characterization to genus and species level (Yao *et al.*, 1996). The PCR-based Deoxyribonucleic Acid (DNA) fingerprinting techniques are rapid, having moderate-to-high sensitivity and are cost-effective compared to DNA sequencing and metagenomics. In addition, this fingerprinting approach allows for multiple samples to be analysed at the same time (Cellier *et al.*, 2020). Molecular fingerprinting via DNA has been demonstrated to distinguish between microbial communities, however, this approach may not provide phylogenetic information in some instances (Di Giovanni *et al.*, 1999; Maldonado and Quintana, 2009; Singh *et al.*, 2019). Overall, DNA fingerprinting techniques offer better accuracy than the morphological/phenotypic methods, which are labour-intensive and time-consuming.

Furthermore, PCR techniques have been successfully used to characterize microbial diversity. This includes techniques such as ribosomal RNA genes (16S rDNA), enterobacterial repetitive intergenic consensus (ERIC), random amplified polymorphic DNA (RAPD) and the non-coding internal transcribed spacers (ITS) region (Redondo *et al.*, 2009; Sadeghian *et al.*,

2016; Sallman *et al.*, 2018; Farzi *et al.*, 2019; Wassermann *et al.*, 2019b). Therefore, the objectives of this study were: (a) to evaluate the antimicrobial efficiency of electrolyzed water treatments for reducing microbial load on the surface of the apple fruit in comparison to conventional practice; (b) to compare the shift in microbial community on the surface of the apple fruit during cold storage; and (c) to identify the major/predominant microbial community on the apple fruit surface before and after treatment with EW during cold storage.

5.2. Materials and Methods

5.2.1. Plant materials and postharvest treatment

Fresh 'Granny Smith' apples (*Malus domestica*) were harvested at commercial maturity from the Agricultural Research Council (ARC) Elgin Research Farm, Grabouw, South Africa. The maturity index was based on total soluble solids (TSS) = 10.66 °Brix. Harvested fruit was transported in cool trucks from the farm to the Post-harvest iQ Laboratory, ARC Infriutec-Nietvoorbij, Stellenbosch, where they were sorted upon arrival. Only mature, healthy and unblemished fruit were selected and stored at 0.5 °C, without any pre-treatment for 3 months prior to pre-treatments and quality analysis.

Electrolyzed water was generated using the ELA-12 000ANW system (ECA Technologies, Envirolyte, South Africa). The AIEW was generated by electrolysis of hydrochloric acid in the range (0.05 %), sodium chloride (0.26 %) and water (99.69 %). The electrolyte flow passed through an electrolytic cell at a rate of 2 mL min^{-1}, and current of 3.8–3.9 volts (V) with an amperage of 10 amperes (A). The AIEW obtained consisted of available chlorine concentration (ACC) of 500 mg L^{-1}, ORP > -800 mV, and pH = 11-13. The AIEW was collected at low temperature (4 °C) and used immediately in the study. Food grade sodium hypochlorite (NaOCl, 11.5% M/V) solution was purchased from Protea Chemicals, Sandton, South Africa**.** All treatment solutions were diluted to desired concentrations prior to dipping the apples and non-treated samples were considered as controls. The dipping duration selected was based on the average pack-house processing time for apples. Description of treatments and abbreviations as used further in this study are presented in Table 5.1. All treated and non-treated (control) batches were stored at 5 °C with 90 ± 2% RH for 21 d and samples were taken for analyses on days 0, 7, 14, and 21.

Table 5.1. Description of treatments and abbreviations used in the study.

Treatment(s)			Treatment(s) abbreviation
Active compound	**Concentration (ppm)**	**Dipping duration (min)**	
NaOCl	200	10	C1
NaOCl	200	15	C2
AIEW	300	10	EW1
AIEW	300	15	EW2
AIEW	200	10	EW3
AIEW	200	15	EW4
Non-treated	-	-	Control

5.2.2. Microbial analysis and morphological characterization

Total plate count was performed to determine total aerobic mesophilic bacteria, and yeasts and moulds of the whole apple fruit surface as described by Caleb *et al.* (2013). The surface area (4.53 ± 95.7 cm^2) of the 'Granny Smith' apples (n = 5) was estimated based on the approach reported by Clayton *et al.* (1995). Each whole apple fruit was submerged in physiological saline (PS, 0.85%) solution in a 600 mL beaker and kept on an orbital shaker at 120 rotations per minute (rpm) for an hour at 28 °C. Threefold serial dilutions were prepared using 1.0 mL of the inoculum PS into 9.0 mL of PS. In order to enumerate the microbial load, 1.0 mL of each dilution was plated, in triplicate onto the respective media. Total aerobic bacteria were enumerated using plate count agar (PCA), while for yeasts and moulds potato dextrose agar (PDA) was used. Plates were incubated for 3-5 days at 25 °C for yeasts and moulds, and 2 days at 30 °C for aerobic-mesophilic bacteria. The results were expressed as log colony forming unit per centimetre of sample (Log CFU cm^{-2}).

After plate count was completed, each typical colony was differentiated based on their morphological features based on macroscopic features on solid media, bacterial colonies were assessed on the following criteria: colour, elevation and margin of colony, opacity, consistency, and surface of the colony (Table 3). Microscopic features were determined through Gram staining according to Salo and Novero (2020). After preliminary identification, pure colonies were subcultured and freeze cultures prepared. Freeze cultures were prepared in a 1:1 ratio, i.e., 900 µL of 60% glycerol and 900 µl of the isolated culture, under sterile conditions and thereafter stored at -80°C at the ARC Infruitec-Nietvoorbij Gene Bank, Stellenbosch until further analysis.

5.2.3. Isolation and inoculum preparation

Freeze cultures of bacteria and yeast and mould obtained from the Genbank were spotted onto De Man, Rogosa and Sharpe (MRS) and Yeast Mould (YM) agar, respectively. The YM agar consist of yeast extract, malt extract, peptone, glucose, agar, distilled water and chloramphenicol (0.1 g) dissolved in 1 mL ethanol (96% v/v), while MRS agar, contains peptone, meat extract, yeast extract, glucose, sodium acetate, triammonium citrate, magnesium sulphate, manganese sulphate, dipotassium phosphate, tween 80, agar, distilled water and 0.1 g of natamax, dissolved in 1 mL distilled water. Both plates were incubated for 3-5 days at 25 °C for yeasts and moulds and 2 days at 30 °C for aerobic-mesophilic bacteria. Single culture colonies from the spotted plates were inoculated into YM broth (for the cultivation of yeast and mould), and into MRS broth (for bacteria). Both broths were incubated for 3-5 days at 25 °C for yeasts and moulds, and MRS broths for 2 days at 30 °C for aerobic-mesophilic bacteria.

5.2.4. Genomic extraction

Total genomic deoxyribonucleic acid (DNA) from yeast and bacteria were isolated using the lithium acetate (LiOAc)-sodium dodecyl sulfate (SDS) lysis extraction method as described by Pulpipat *et al.* (2013). Bacterial cell pellet was suspended in 200 µL of 200 mM LiOAc 1% SDS solution and incubated for 15 min at 70 °C. A volume of 500 µL of 96% ethanol was added for DNA precipitation. Samples were mixed briefly, and DNA was collected by centrifugation at 13,800 g for 5 min. The supernatant was removed, and the DNA pellet was washed with 500 µL of 70% ethanol and centrifuged again at 13,800 g for 5 min. Thereafter, the DNA pellets were dried at 26 °C and then suspended in a 30 µL Tris-EDTA (TE) buffer. The cell debris was removed by centrifugation at 13800 g for 15 s and the supernatant was used for PCR template.

5.3. Molecular characterisation of the Isolates

5.3.1. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR)

The total genomic material was amplified using ERIC1 and ERIC2 (Table 5.2) to identify the bacterial population. The ERIC-PCRs were carried out in a 25 µL reaction volume that contained 2 µL rDNA templates according to Sallman *et al.* (2018). To each 25 µL reaction mixture: a 10 × amplification buffer with Mg (Separation Scientific, Honeydew, South Africa); a 2.5 mM dNTP (Separation Scientific, Honeydew, South Africa); 25 mL $MgCl_2$; ERIC 1R and ERIC2 oligonucleotide primers (IDT, Whitehead Scientific, South Africa); 20 mg mL^{-1} bovine serum albumin (Sigma Chemical Co., St Louis, MO); and 5u/µL super-therm polymerase (Separation Scientific, Honeydew, South Africa) and addition of distilled water. The PCR was

performed using a programmable thermal cycler with a heated lid (Techni, 3Prime, Lasec, South Africa). Amplification conditions were as follows: initial denaturation at 95 °C for 7 min; samples held at 75 °C for 1 min; followed by 35 cycles of denaturation at of 94 °C for 5 min and 35 cycles of 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 5 min followed by a final extension of 72 °C for 10 min. The samples were maintained at 4 °C until analysis by gel electrophoresis.

5.3.2. Random amplified polymorphic DNA (RAPD) analysis

The RAPD-PCRs were carried out in a 25 µL reaction volume that contained 2 µL rDNA templates according to a method by Farzi *et al.* (2019). The RAPD-PCRs were amplified with RAPD1283 primer (Table 5.2). The following was added to each 25 µL reaction mixture: a 10 × amplification buffer with $Mg2^{++}$ (Separation Scientific, Honeydew, South Africa); a 2.5 mM dNTP (Separation Scientific, Honeydew, South Africa); 25 mL $MgCl_2$; primer RAPD1283 (IDT, Whitehead Scientific, South Africa); 20 mg mL^{-1} bovine serum albumin (Sigma Chemical Co., St Louis, MO); and 5 u/µL Super-Therm polymerase (Separation Scientific, Honeydew, South Africa) and addition of distilled water. The PCR was performed using a Programmable Thermal Cycler with a heated lid (Techni, 3Prime, Lasec, South Africa). The thermal conditions were initial denaturation step of 95 °C for 4 min, followed by 30 cycles (denaturation at 94 °C for 30 s, annealing at 38 °C, extension at 72 °C for 2 min with a final extension of 72 °C for 8 min) and a final hold at 10 °C for 10 s. The samples were maintained at 4 °C until analysis by gel electrophoresis.

5.3.3. Internal transcribed spacers (ITS)

The various fungal rDNA were amplified by PCR according to a method described by Siavoshi *et al.*, (2018) with primers ITS1 and ITS4 (Table 5.2). Polymerase Chain Reactions were carried out in volumes of 50 µL comprising: 1 µL of the DNA template, 10 × amplification buffer with Mg (Separation Scientific, Honeydew, South Africa); a 2.5 mM dNTP (Separation Scientific, Honeydew, South Africa); 25 mL $MgCl_2$; ITS1 and ITS4 primer (IDT, Whitehead Scientific, South Africa); 20 mg ml^{-1} bovine serum albumin (Sigma Chemical Co., St Louis, MO); 5 u/µL super-therm polymerase (Separation Scientific, Honeydew, South Africa) and addition of distilled water. Reactions were performed using a programmable thermal cycler with heated lid (PTC-100, Techni, 3Prime, Lasec, South Africa), with the following conditions: initial denaturation step of 95 °C for 4 min, followed by 30 cycles (denaturation at 94 °C for 30 s, annealing at 38 °C, extension at 72 °C for 2 min with a final extension of 72 °C for 8 min) and a final hold at 10 °C for 10 s. The samples were maintained at 4 °C until analysis by gel electrophoresis.

5.3.4. Amplification of 16s Ribosomal DNA (rDNA)

The various bacterial rDNAs were amplified by PCR according to a method by Konecka and Olszanowski (2019) with primers 16SD and 16SR (Table 5.2). The 16s rDNA PCRs were carried out in a 50 µL reaction volume that contained 2 µL of the DNA template, a 10 × amplification buffer with Mg (Separation Scientific, Honeydew, South Africa); a 2.5 mM dNTP (Separation Scientific, Honeydew, South Africa); 16sd and 16sr primers (IDT, Whitehead Scientific, South Africa); 25 mL $MgCl_2$; 5 u/µL super-therm polymerase (Separation Scientific, Honeydew, South Africa). Reactions were performed using the programmable thermal cycler with heated lid (PTC-100, Techni, 3Prime, Lasec, South Africa), with the following conditions: initial denaturation at 94 °C for 30 s followed by 30 cycles at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min and a final extension of 72 °C for 8 min. Thereafter, samples were maintained at 4 °C until analysed by gel electrophoresis.

Table 5.2. Oligonucleotide primers used in this study.

Primer	Sequence (5′to 3′)	Reference
RAPD1283	5'-GCG ATC CCC A-3'	Farzi *et al.*, 2019
ERIC1	5´-ATGTAAGCTCCTGGGGATTCAC-3´	Sallman *et al.*, 2018
ERIC2	5´-AAGTAAGTGACTGGGGTGAGCG -3´	Sallman *et al.*, 2018
ITS1	5′-TCCGTAGGTGAACCTGCGG-3′	Siavoshi *et al.*, 2018
ITS4	5-TCCTCCGCTTATTGATATGC-3′	Siavoshi *et al.*, 2018
16SD	5′-GGTACCYACAGAAGA-AGTCC-3′	Konecka and Olszanowski, 2019
16SR	5′-TAGCACTCATCGTTTACAGC-3'	Konecka and Olszanowski, 2019

5.4. Analysis by electrophoresis

The presence and quality of the extracted genomic DNA and all PCR amplicon samples was analysed using a 1.5% agarose gel containing 0.01% ($^{v}/_{v}$) −1 ethidium bromide at 4°C. Loading dye (5 µL) was added to each genomic DNA extract and all PCR amplicons, thereafter 10 µL of each sample was inoculated into separate wells of the gel and electrophoresed for 90 min at 90 V (VACUTEC Thermocycler, South Africa). Thereafter, the gel images were captured using a Gel Doc image analyser (UVTEC Image Analyser, United Kingdom) to visualise chromosomal banding patterns.

5.5. Statistical Analysis

Factorial analysis of variance (ANOVA) was used to elucidate the impacts of treatments, dipping time and storage duration on microbial load on the surface of cv. 'Granny Smith' apples at 95% confidence interval using Statistica Software (version 13, StatSoft Inc. TIBCO Software Inc., USA). Post-hoc test (Duncan multi-range test) was used to determine the difference between mean values. All analyses were conducted in triplicate and results were presented as mean (n = 3) ± standard deviation.

5.6. Results and Discussion

5.6.1. Morphological characterization

Throughout the entire storage period, a total of 56 morphologically distinct bacteria colonies were isolated based on parameters listed in Table 5.3. While only ten distinct fungal colonies were isolated and characterized based on colony structure. Based on the plate and isolates the predominant microbial population after treatment with electrolyte water on the apple fruit surface were bacteria. Similar results indicating that the microbiota on the surface of apple fruit is predominately bacterial as compared to fungal was reported by Wassermann *et al.* (2019b). In a similar study using a different post-harvest treatment, Wassermann *et al.* (2019a) indicated that microbiota on the surface of apple fruit was predominately bacterial as compared to fungal after treatments. The lower fungal diversity on the apple fruit surface could be attributed to the soil nutrient content (Gu *et al.*, 2020), water activity of the fruit surface (Maffei *et al.*, 2016), orchard production strategies (Shen *et al.*, 2018b) and the need of bacterial attachment to the external surface of fungi (Steffan *et al.*, 2020). Other studies by Abdelfattah *et al.* (2021b) and Bösch *et al.* (2021) focused on the global analysis of the apple fruit microbiome after harvest which indicated that the predominant microbial population was that of fungi.

The major/most common bacteria isolates found on the apple surface consisted of cream and yellowish smooth textured, white irregular shaped and cream-white rough textured with irregular shaped colonies (Table 5.3). Compared to the non-treated control samples, ≈ 2 Log reduction in total aerobic mesophilic bacteria (from 4.1 Log CFU cm^2 to 2.2 Log CFU cm^2) and < 1 Log reduction for yeast and mould (from 3.9 Log CFU cm^2 to 2.7 Log CFU cm^2) count was recorded at the end of 21 days storage (Table 5.4). Samples treated under EW4 showed the greatest reduction for total aerobic mesophilic bacteria compared to other treatments and remained significantly lower than the control sample at the end of storage. Based on Gram staining, a total of four Gram-positive and four Gram-negative bacteria were confirmed (Table 5.3). In addition, the microscopic morphological assessments showed that the bacteria shapes were bacilli and coccobacilli intermediate between cocci (round shaped) and bacilli (rod shaped). Figure 5.1 shows a microscopic representation of the bacteria isolates.

Table 5.3. Morphological characteristics of bacteria isolated from electrolyte treated ‘Granny Smith’ apples.

Colony	Colony morphology				Gram classification	Shape	Arrangement
	Colour	Colony Form	Texture	Appearance			
1	Cream-yellowish	Circular	Smooth	Mucoid	Gram-negative	Rod-shaped	Chains
2	White	Irregular	Smooth	Opaque	Gram-negative	Rod-shaped	Chains
3	Cream	Circular	Wrinkled	Dry	Gram-positive	Cocci-shaped	Single
4	Yellow	Circular	Slimy	Mucoid	Gram-positive	Rod-shaped	Chains
5	Cream-white	Irregular	Rough	Dry	Gram-negative	Rod-shaped	Chains
6	Brown	Circular	Smooth	Opaque	Gram-negative	Rod-shaped	Chains
7	Purple-pink	Circular	Smooth	Mucoid	Gram-negative	Cocci-shaped	Single
8	Orange-yellow	Circular	Smooth	Mucoid	Gram-negative	Rod-shaped	Chains

Adapted from Salo and Novero (2020).

Table 5.4. Effect of different treatments on the cv. 'Granny Smith' apple surface microbial load (total aerobic mesophilic bacteria, yeasts and moulds) expressed as log CFU/cm^2.

Treatment(s)	Storage duration (Days)	Aerobic Mesophilic Bacteria log CFU/cm^2	Yeast and Mould Log CFU/cm^2
C1	0	$3.74^{b} \pm 0.09$	$3.74^{c} \pm 0.04$
	7	$3.41^{bc} \pm 0.21$	$3.14^{e} \pm 0.01$
	14	$3.20^{c} \pm 0.09$	$2.46^{fg} \pm 0.15$
	21	$3.17^{cd} \pm 0.29$	$3.48^{d} \pm 0.18$
C2	0	$3.74^{b} \pm 0.09$	$3.74^{c} \pm 0.04$
	7	$3.35^{cd} \pm 0.23$	$2.67^{fg} \pm 0.18$
	14	$3.13^{cd} \pm 0.20$	$2.85^{ef} \pm 0.20$
	21	$2.89^{def} \pm 0.37$	$3.15^{e} \pm 0.02$
EW1	0	$3.74^{b} \pm 0.09$	$3.74^{c} \pm 0.04$
	7	$2.80^{e} \pm 0.17$	$2.69^{f} \pm 0.21$
	14	$3.09^{cde} \pm 0.36$	$2.95^{ef} \pm 0.20$
	21	$3.16^{cde} \pm 0.36$	$3.16^{e} \pm 0.04$
EW2	0	$3.74^{b} \pm 0.09$	$3.74^{c} \pm 0.04$
	7	$2.73^{e} \pm 0.08$	$2.61^{fg} \pm 0.11$
	14	$3.11^{cd} \pm 0.23$	$2.66^{fg} \pm 0.21$
	21	$3.40^{bc} \pm 0.21$	$2.61^{f} \pm 0.11$
EW3	0	$3.74^{b} \pm 0.09$	$3.74^{c} \pm 0.04$
	7	$2.81^{de} \pm 0.18$	$2.26^{g} \pm 0.24$
	14	$2.68^{def} \pm 0.33$	$2.62^{fg} \pm 0.41$
	21	$2.98^{de} \pm 0.22$	$2.66^{fg} \pm 0.22$
EW4	0	$3.74^{b} \pm 0.09$	$3.74^{c} \pm 0.04$
	7	$2.24^{f} \pm 0.32$	$2.74^{fg} \pm 0.24$
	14	$3.18^{cde} \pm 0.42$	$2.57^{fg} \pm 0.23$
	21	$2.44^{ef} \pm 0.42$	$2.66^{fg} \pm 0.34$
Control	0	$3.74^{b} \pm 0.09$	$3.74^{c} \pm 0.04$
	7	$4.15^{a} \pm 0.04$	$4.03^{bc} \pm 0.22$
	14	$4.16^{a} \pm 0.02$	$4.26^{b} \pm 0.01$
	21	$3.93^{ab} \pm 0.19$	$4.43^{a} \pm 0.03$

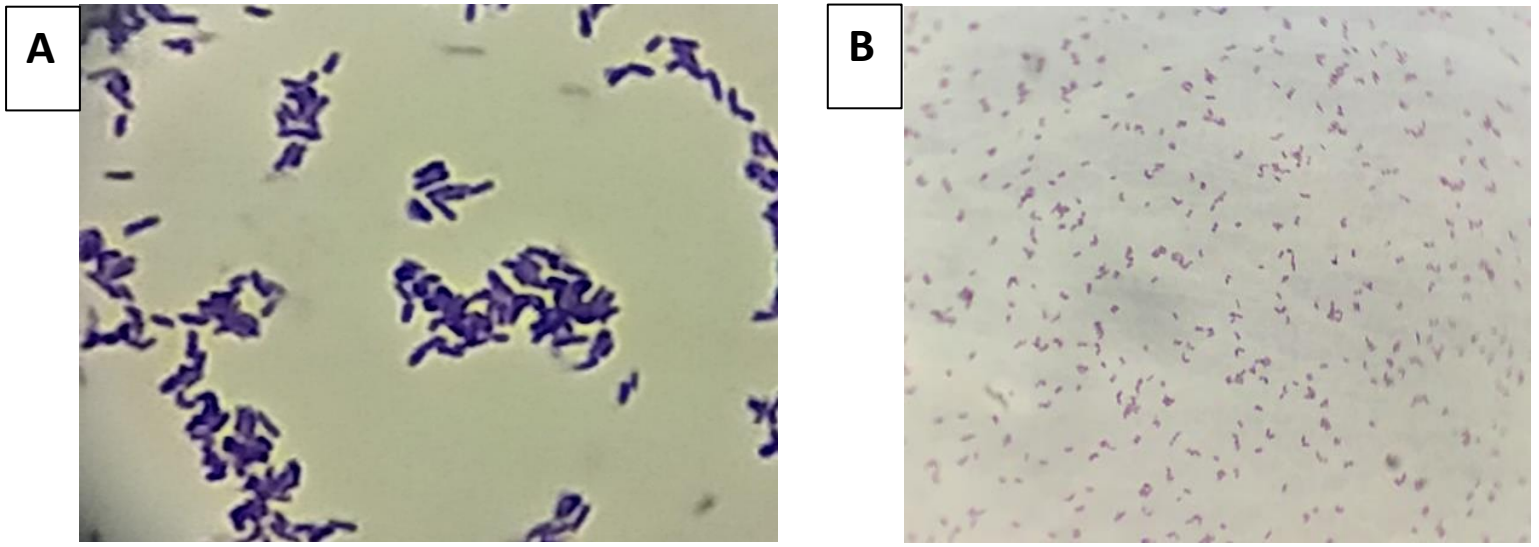


Figure 5.1. Photo micrographs of (A) Gram- positive bacteria, and (B) Gram- negative bacteria isolated from electrolyte water treated cv. ‘Granny Smith’ apple fruit at 1000X magnification.

5.6.2. Molecular bacterial characterization

5.6.2.1. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) analysis

The amplicon patterns obtained via ERIC PCR are shown in Figure 5.2. Amplicons of varying base pair (bp) sizes ranging from 100-3000 bp were observed across the selected bacteria genomic DNA. Over fifty six (56) bacterial genomic DNA extracts were selected from all the treatment types between day 0 and 21. Of the 56 bacterial genomic DNA, 19 of them were characterized as similar based on ERIC PRC analysis, this includes bacterial genomic DNA extracts with 100-3000 bps (L: 8, 12, 15, 20, 21, 24 and 26); (L: 9, 8 and 23); (L: 3, 1 3, 22 and 30); (L: 4, 15, and 20) and (L: 5 and 14). The similarities could have meant that the bacterial genomic DNA extracts were that of closely related species and based on this, the bacterial genomic DNA were then grouped according to their similarities and differences. It has been proven that ERIC-PCR has the ability to discriminate between strains of the same or closely related species (Aljindan *et al.*, 2018; Bakhshi *et al.*, 2018). This study clearly showed that ERIC-PCR was able to discriminate between members of the same or closely related species. Therefore, using ERIC-PCR, only 27 out of the 56 isolates resulted in the generation of different ERIC-PCR genotypes.

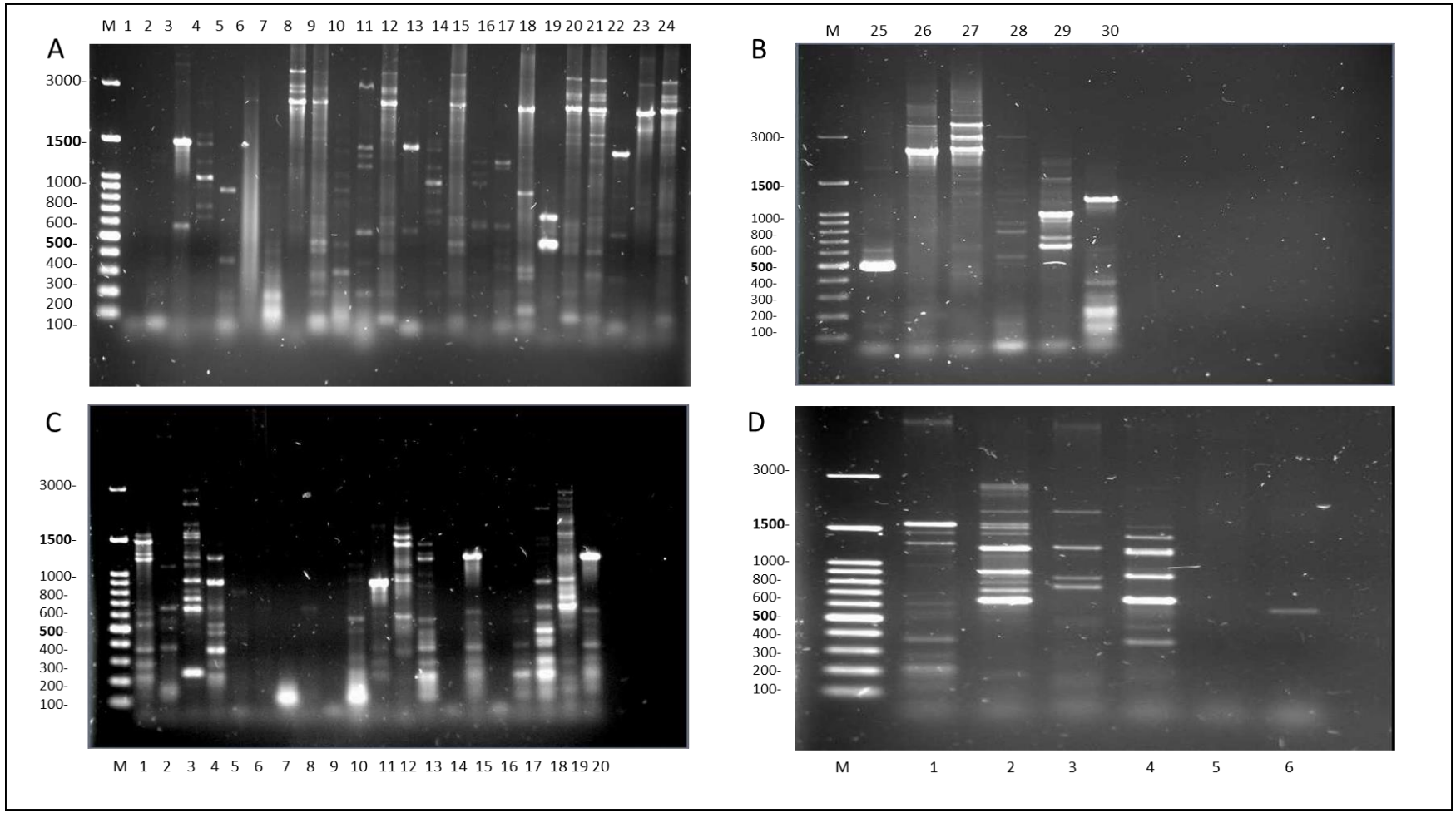


Figure 5.2. (A and B) ERIC PCR fingerprints of test bacterial isolates from week 1 of post-harvest storage. Lane M: DNA marker, lanes (1-30) **(C)** ERIC PCR fingerprints of test bacterial isolates from week 2 of post-harvest storage. Lane M: DNA marker, lanes (1-20) **(D)** ERIC PCR fingerprints of test bacterial isolates from week 3 of post-harvest storage. Lane M: DNA marker, lanes (1-6)

5.6.2.2. 16s rRNA typing

Following amplification of the 16s rRNA gene fragment, an analysis of the amplified products on agarose gel revealed that the amplification took place in 16 bacterial isolates to generate 1500bp amplicons (Figure 5.3a). Sequence analysis of the amplicons showed a high level of similarity between the test sequences and the sequences at the GenBank (Table 5.5). In the first week after treatment and post-harvest storage, there was growth on the surfaces of cv. 'Granny Smith' apples among all treatment types and the control.

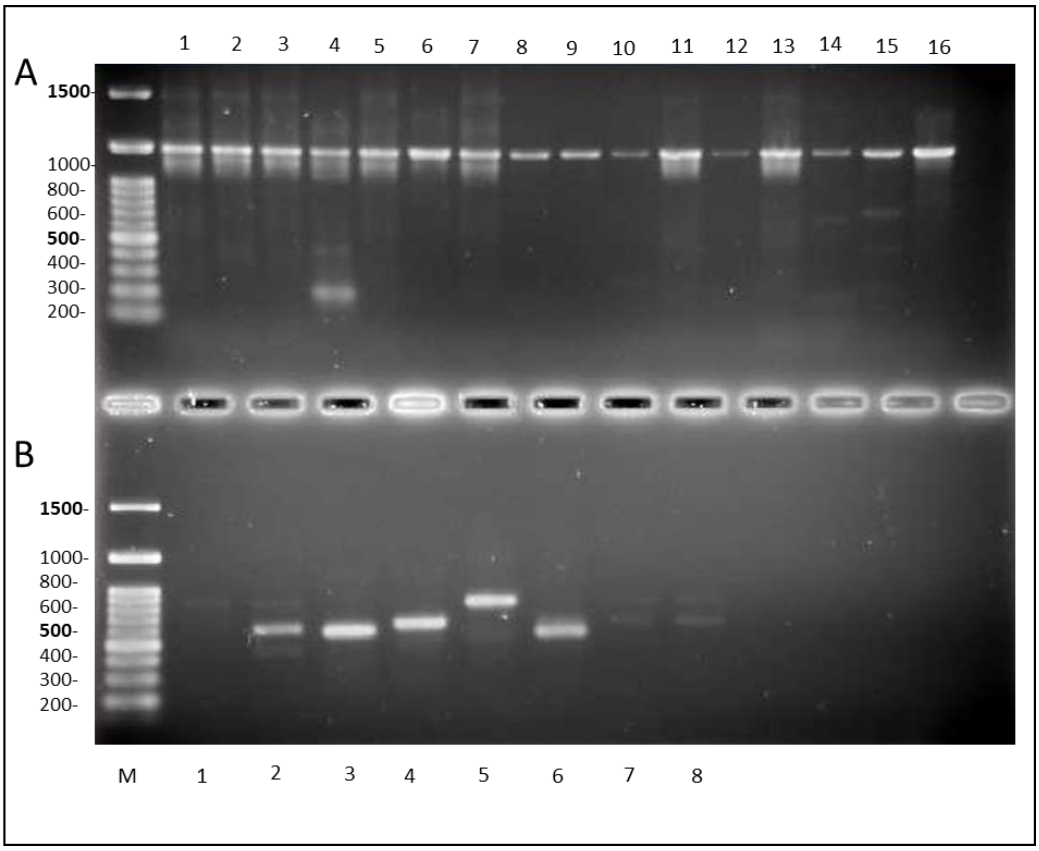


Figure 5.3. PCR products of 16S and 16D primer of band size 100-1500bp, electrophoresis on 1.5% agarose at 90 volt/ cm2 for 1:30 hours with UV visualization. Lane M: DNA ladder (100bp), lanes (1-16) PCR products (B) PCR products of ITS1 and ITS4 primer of band size 100-1500bp, electrophoresis on 1.5% agarose at 90 volt/ cm^2 for 1:30 hours with UV visualization. Lane M: DNA ladder (100bp), lanes (1-8) PCR products.

Table 5.4. Major microbiota found on the surface of 'Granny Smith' apples across all treatments during 21 days post-harvest storage at 5 °C using ITS and 16S rDNA sequences.

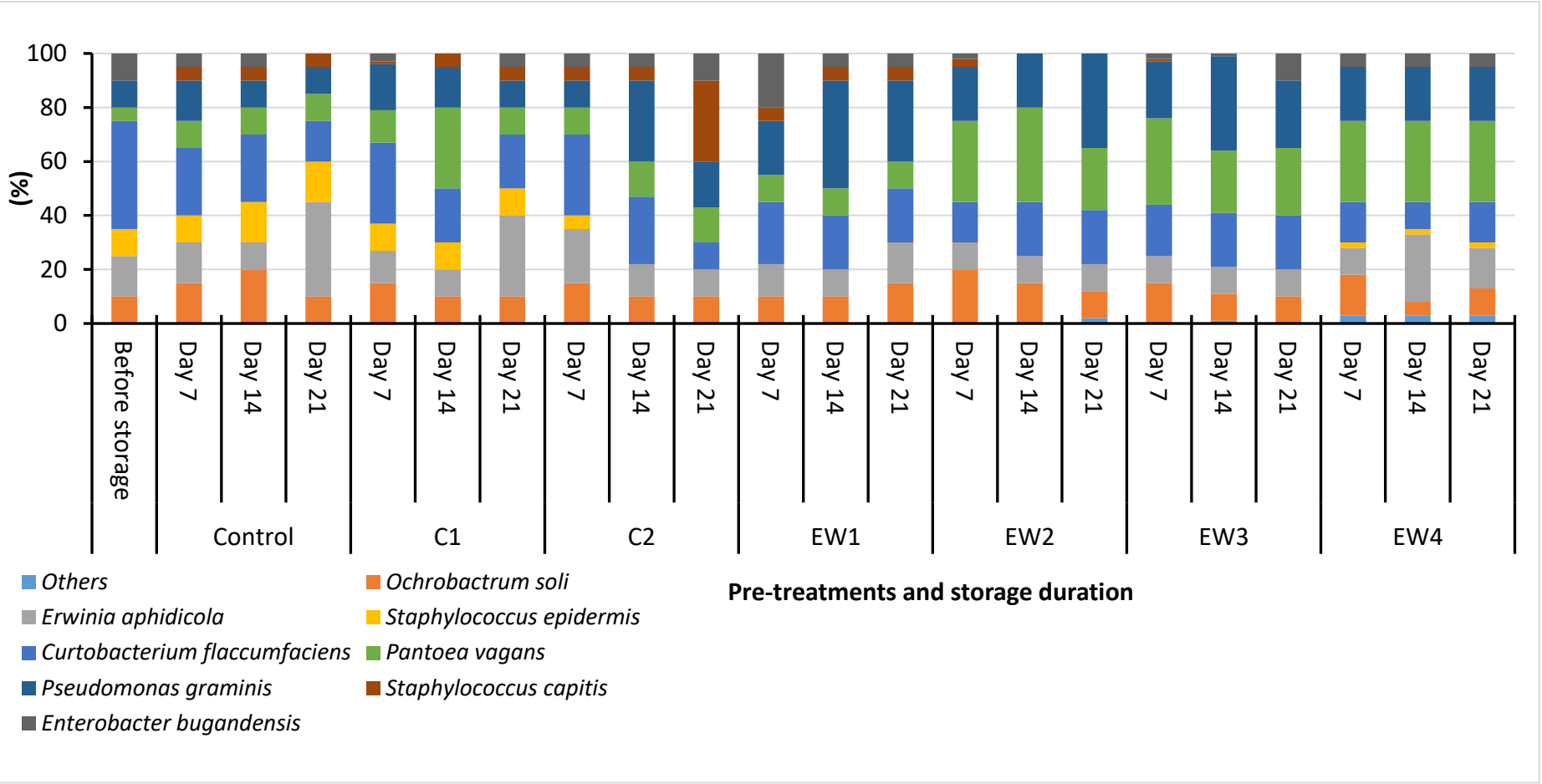
Major microbiota	Species	Sequence length (bp)	Identity (% accuracy)	GenBank Accession No.	Reference
Mould/Yeast					
	Rhodotorula nothofagi	586	99	KY104889.1	Vu *et al.*, 2016
	Aspergillus inuii	821	95	MH856954.1	Vu *et al.*, 2019
	Debaryomyces hansenii	614	100	KY103230.1	Vu *et al.*, 2016
	Phialemoniopsis curvata	527	96	AB278180.1	Yaguchi *et al.*, 2006
Bacteria					
	Ochrobactrum soli	1229	95	NR_169364.1	Choi *et al.*, 2020
	Erwinia aphidicola	1072	94	LT548976.1	Lopez-Fernandez *et al.*, 2018
	Staphylococcus epidermidis	1252	91	CP035288.1	Argemi *et al.*, 2018
	Curtobacterium flaccumfaciens	1123	85	MN687837.1	Musa, 2019
	Pantoea agglomerans	1179	84	CP077366.1	Sproer *et al.,* 2021
	Pseudomonas graminis	1214	94	LN551925.1	Janssen *et al.*, 2015
	Enterobacter bugandensis	1217	92	KI911561.1	Das *et al.,* 2013
	Staphylococcus capitis	1240	97	MF678924.1	Kosecka-Strojek *et al.*, 2019

As represented in Figure 5.4, eight different types of bacteria were isolated from the surface of the apple fruit and identified in all treatments during 21 days of storage at 5 °C as follows: *Ochrobactrum soli*, *Erwinia aphidicola*, *Enterobacter bugandensis*, *Staphylococcus epidermidis, Curtobacterium flaccumfaciens, Pantoea agglomerans, Pseudomonas graminis* and *Staphylococcus capitis*. The bacterial community composition was affected by treatment type (Figure 5.4). Furthermore, cold storage and storage duration were found to influence the bacterial diversity. For instance, at the end of day 7 storage the dominant microbiota notably shifted in comparison to the before storage profile. *Curobacterium flaccumfaciens* was the most dominant bacteria found before storage and after postharvest treatments. As the storage progressed, EW4 was the most effective in decreasing *Curtobacterium flaccumfaciens* bacterial count. *Curtobacterium flaccumfaciens* is known to survive only a couple of weeks as free bacteria in the soil and is normally dispersed via agricultural practices such as, planting saved seed and through farm equipment (Gonçalves *et al.*, 2017; Nascimento *et al.*, 2020). Similarly on day 21 *Pantoea agglomerans* were most dominant. *Pseudomonas graminis* which was found before storage and after postharvest treatments (control, C1, C2, EW1, EW2, EW3 and EW4) throughout 21 days postharvest storage at 5 °C is a native strain from whole apple surfaces. Some strains of *P. grammis such as Pseudomonas graminis* CPA-7, are identified as an effective biocontrol agent against *Listeria monocytogenes*, *Salmonella enterica* and *Escherichia coli* O157:H7 on fresh-cut fruit (Quaglia *et al.*, 2011; Alegre *et al.*, 2013a,b). The presence of *P. graminis* on the apple surface in this study could have possibly inhibited the growth of other bacteria such as *S. capitis* and *S. epidermidis* which were found after storage and postharvest treatment with C1 and C2. Humans are likely to be the main sources or vehicles of transmission of *S. capitis* (Cui *et al.*, 2013), hence they were only found after day 7 of postharvest storage across the control, C1, C2 and EW1 treatments.

The composition of the postharvest microbiome of apple can also be impacted by bacterial communities present in pack houses that process fruit for market and storage after harvest. A recent study reported substantial differences in both the composition and diversity of the environmental microbiota present in different apple fruit processing facilities, and revealed that the presence of *Pseudomonadaceae* families was associated with an increased occurrence and persistence of the foodborne pathogen *Listeria monocytogenes* (Tan *et al.*, 2019). Previous studies by Wassermann *et al.*, (2019b) found the overall bacterial community of all apple samples, assessed by 16S rRNA gene amplicon sequencing, to contain bacterial phyla, *Rhizobiales* (12%), *Pseudomonadales* (11%), and *Enterobacteriales* (7%); Pseudomonas (11%), *Pantoea* (5%), and *Hymenobacter* (5%) were furthermore highly abundant.

Comparing the treatment types and storage duration, a clear differentiation was observed for all treatment types with EW4 being the most effective in reducing all the bacterial counts. In addition, *Ochrobactrum soli*, *Erwinia aphidicola*, *Enterobacter bugandensis*, *Staphylococcus epidermidis, Curtobacterium flaccumfaciens, Pantoea agglomerans* and *Pseudomonas graminis* were already present before treatment and storage and decreased to lower levels in EW1, EW2 and EW3 storage and, to a lesser extent, in EW4 (Figure 5.4). The results showed a large effect of the treatment type on the amounts of bacteria (Figure 5.4). *S. capitis* and *S. epidermidis* on the other hand were present in low numbers from day 7 of postharvest storage and decreased until the end of the storage period (Figure 5.4). The C1 and C2 treatment did not change the abundance of bacteria compared to the control treatment, while all EW treatments decreased the abundance of bacteria, (Figure 5.4).

Relatively few genera contribute a large portion of the microbiome on fruit and the fruit microbiome changes during storage depending on the storage conditions. In addition, the postharvest treatment regime has an influence on the diversity of the fruit microbiome and on the dynamics of pathogenic bacterial and fungal genera during storage.



*C1= NaOCl, 200 mg/L^{-1} at 10 min; C2= NaOCl, 200 mg/L^{-1} at 15 min; EW1= AIEW, 300 mg/L^{-1} at 10 min; EW2= AIEW, 300 mg/L^{-1} at 15 min; EW3= AIEW, 200 mg/L^{-1} at 10 min, EW4= AIEW, 200 mg/L^{-1} at 15 min

Figure 5.4. Culturable bacterial microflora associated with treated and non-treated apple fruit surface during storage at 5 °C for 21 days.

5.7. Fungal characterization

5.7.1. Random Amplification of the Polymorphic (RAPD-PCR)

Using RAPD-PCR typing with the primer RAPD1283, out of the approximately 30 fungal isolates only a total of 10 distinct band patterns were identified, across all treated and non-treated 'Granny Smith' apple fruit surfaces during the storage period (Figure 5.5). Bands obtained on each gel ranged from 100 to 3000 bp. The RAPD analysis compares the whole chromosomal DNA displaying bands of genotypically specific values, and is fast and trust-worthy (Molnár *et al.*, 1993). It uses short (5–10 mer) oli-gonucleotide primers with arbitrary sequences at low annealing temperatures that hybridize at loci distributed randomly throughout the genome, allowing the amplification of polymorphic DNA fragments (Lathar *et al.*, 2010). These patterns, based on the RAPD-PCR typing analyses, indicate that the various yeasts isolated from electrolyte water treated cv. 'Granny Smith' apples are different from each other based on the amount of polymorphic bands produced.

Pianzzola *et al.*, (2004) recovered fourteen isolates of *Penicillium expansum* from rotten apples with blue mould symptoms. RAPD primer gave reproducible RAPD patterns, with 13% being the highest difference in band presence between repetitions for the same isolate. Two different and very homogeneous patterns were revealed for natural isolates which corresponded to those of *P. expansum* and *P. solitum* type strains. The RAPD band patterns corresponding to *P. expansum* and *P. solitum* isolates showed up to 78% similarity, whereas those corresponding to the closest related species (*P. expansum* and *P. viridi-catum*) showed similarity levels of about 68%. RAPD analysis proved to be an objective, rapid, and reliable tool to identify *Penicillium spp*. involved in the blue mould of apples. Wei *et al.*, (2017) identified and characterized yeasts on apples for their potential cider-making performance using RAPD-PCR typing. More than 71 different yeast species belonging to 24 different genera were observed following RAPD-PCR sequencing. Younus (2018) isolated *Saccharomyces cerevisiae* present on different fruits (apple, plum, dates, and peach) and performing RAPD analyses. Based on RAPD assay the data developed from the PCR analysis demonstrated that some primers generate several bands, while others generate only a few bands. A total of six RAPD primers were used to study the genetic differences between five *S. cerevisiae* isolates, and amplified 111 bands, of which 101 bands were polymorphic, with average of (1-26) polymorphic bands. In another study by Sharma *et al.* (2019), ten fluorescent *Pseudomonas* species were isolated from normal and replant rhizosphere soil samples of apple. Random Amplified Polymorphic DNA analysis was done to observe genetic homogeneity/polymorphism among 10 isolates of fluorescent *Pseudomonas* species. Phylogenetic analysis of RAPD profiles of ten strains of *Pseudomonas spp.* with four random

10-base oligonucleotide primers exhibited significant genetic polymorphism ranging from 0.17 to 0.67%. Wei *et al.* (2017) identified and characterized yeasts on apples for their potential cider-making performance using RAPD-PCR typing. More than 71 different yeast species belonging to 24 different genera were observed following RAPD-PCR sequencing.

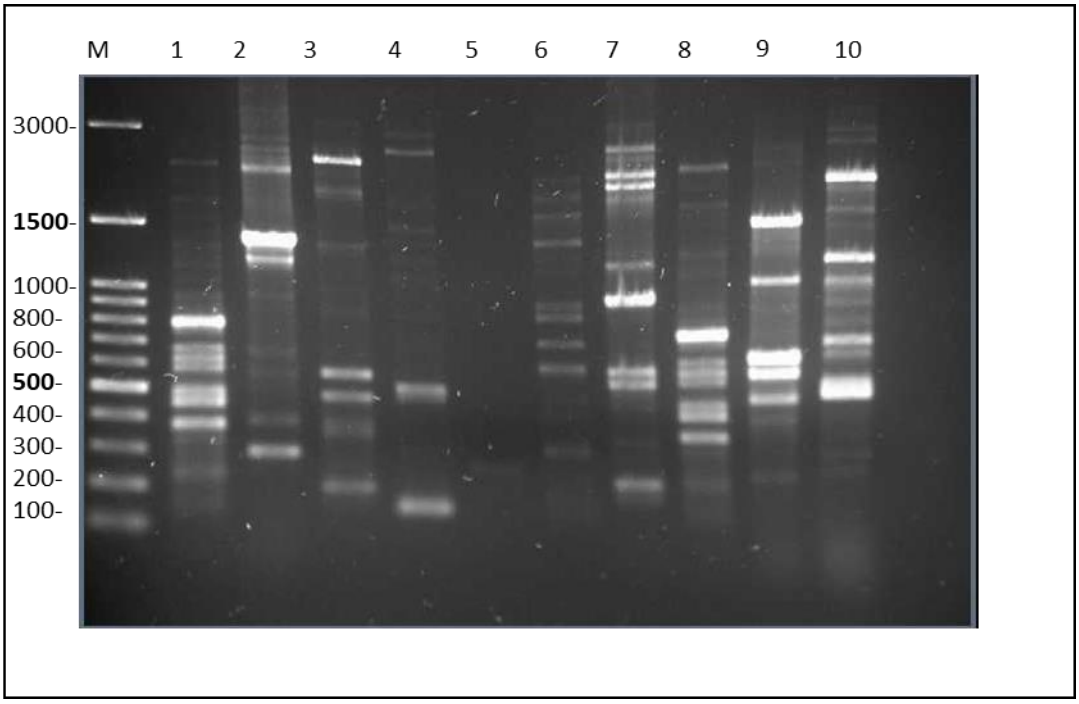


Figure 5. 5. PCR products of RAPD1283 primer of band size 100-3000bp, electrophoresis on 1.5% agarose at 90 volt/ cm2 for 1:30 hours with UV visualization. Lane M: DNA ladder (100bp), lanes (1-10) PCR products.

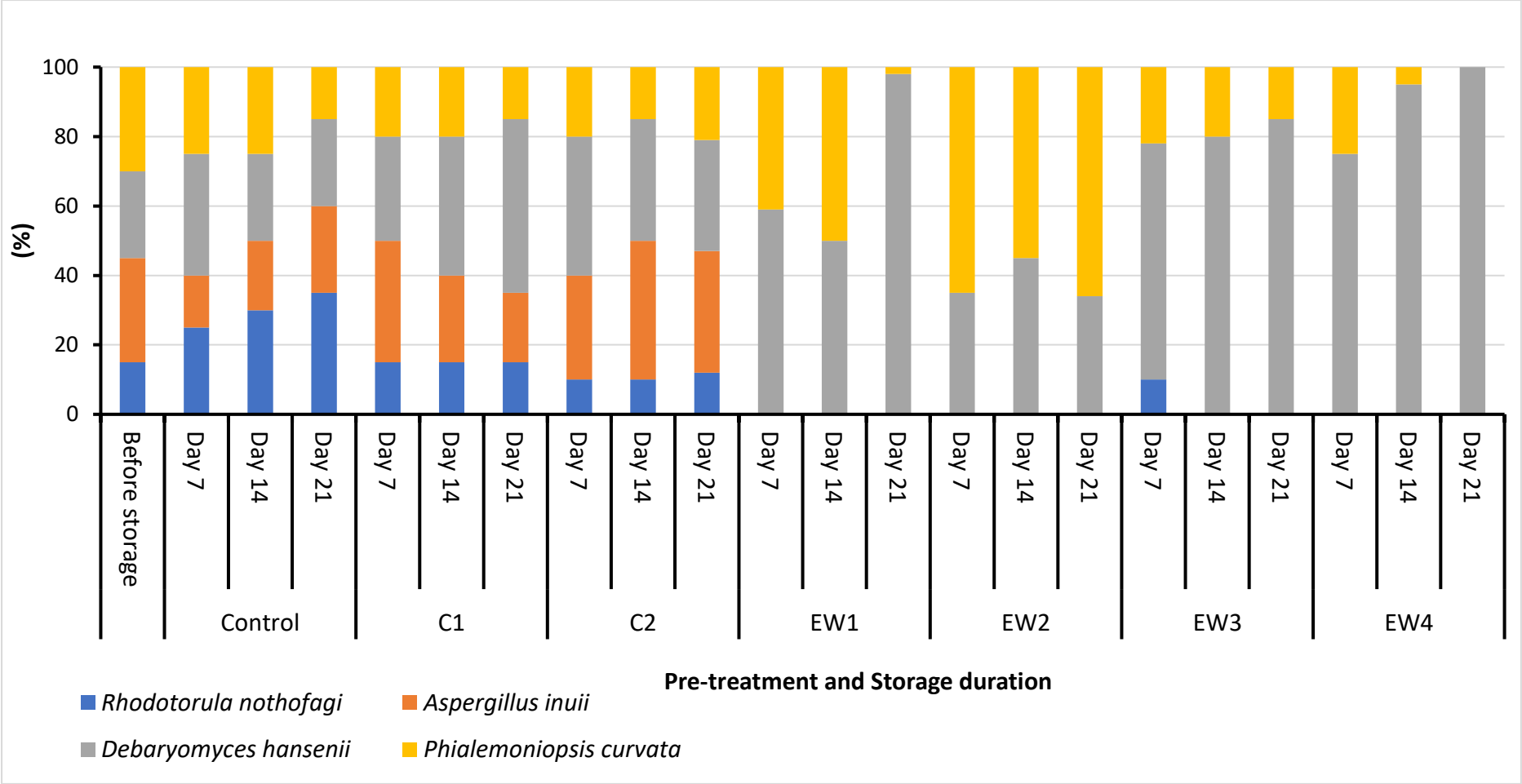
5.7.2. ITS-PCR analysis

The results of ITS1 and ITS4 showed that most of the isolates give a single bundle of DNA on agarose gel (Figure 5.3b). Out of the 10 isolates characterized via RAPD1283, only four isolates could be successfully amplified using the ITS1 and ITS4 regions. This includes *Debaryomyces hansenii*, *Rhodotorula nothofagi*, *Phialemoniosis curvata*, and *Aspergillus inuii* (Table 5.5). The unsuccessful amplification of the other four isolates could be attributed to the conserved nature of ITS1 and -4 region of the fungal isolates (Manter and Vivanco, 2007), as well as the DNA template quantity and quality or the presence of a DNA inhibitor (Taylor and McCormick, 2008).

Grantina-Ievina, (2015) obtained baseline information about apple rot-causing fungi, their incidence during fruit storage and evaluated the fungicide sensitivity of most of isolated fungal species. Fungi were identified according to the morphological characteristics and sequencing of the ITS1-5.8S-ITS2 region. The authors found that in part of the storehouses apple rot caused by *Cadophora luteo-olivacea* was observed and its identity was successfully proved by PCR with ITS1 and ITS 4 primers specific to this species. *Alternaria spp*. and

Cladosporium spp. were detected on a few apples as secondary infection agents. In another study, Shen *et al.*, (2018) characterized the superficial fungi of 18 apple samples before and after cold storage by sequencing internal transcribed spacer 1 (ITS1) sequences. A total of 1,319,679 high-quality ITS reads were obtained from eighteen samples. Respectively, ITS reads of 583,915 (with an average of 64,879 per sample) were obtained from HP samples, and ITS reads of 735,764 (averaged 81,752 per sample) were obtained from CS samples. Recently, Madbouly *et al.* (2020) isolated five endophytic yeasts from cv. 'Golden Delicious' apples and identified the according to their microscopic characteristics *Schwanniomyces vanrijiae*, *Galactomyces geo-trichum*, *Pichia kudriavzevii*, *Debaryomyces hansenii*, and *Rhodotorula glutinis*. The first three isolates showed in vitro inhibitory potential, moreover, they caused a significant inhibition of germination of pathogen conidia by 67.6–89.2%. Identification of these three potent yeasts in addition to *M. fructigena* isolate was confirmed by PCR analysis through amplification of ITS region and PCR products of approximately 650 bp amplified with the ITS1/ITS4 primers and corresponding to the ITS1, 5.8S and ITS2 regions of rDNA were obtained from all isolates.

Fungal community composition was affected by treatment type (Figure 5.6). Furthermore, cold storage and pre-treatment were found to influence the fungal diversity. For instance, at the end of day 7 storage the dominant microbiota notably shifted in comparison to the before storage profile as follows: *Debaryomyces hansenii, Phialemoniosis curvata, Aspergillus inuii* and *Rhodotorula nothofagi* (Figure 7). Similarly on day 21 *Debaryomyces hansenii* and *Phialemoniosis curvata* were most dominant. *Debaryomyces hansenii* has also been recently employed as a biological control against a variety of fruit rot causing diseases, including apples (Peromingo *et al.*, 2019; Madbouly *et al.*, 2020). The phyllo plane yeast *Rhodotorula* spp. found before storage as well as after postharvest treatments (control, C1, C2) has been reported to control *B. cinerea* on geranium seedlings in combination with fungicides (Buck, 2004). The biggest shift in the fungal community composition during storage occurred between 14 and 21 days postharvest storage. The effect of treatment on the fungal community was similarly most evident between EW 1, EW2, EW3 and EW4 treated fruit, with the EW4 exhibiting the highest degree of inactivation (Figure 5.6).



*C1= NaOCl, 200 mg/L^{-1} at 10 min; C2= NaOCl, 200 mg/L^{-1} at 15 min; EW1= AIEW, 300 mg/L^{-1} at 10 min; EW2= AIEW, 300 mg/L^{-1} at 15 min; EW3= AIEW, 200 mg/L^{-1} at 10 min, EW4= AIEW, 200 mg/L^{-1} at 15 min

Figure 5.6. Culturable fungal microflora associated with treated and non-treated apple fruit surface during storage at 5 °C for 21 days.

5.8. Conclusion

Electrolyzed water is an emerging antimicrobial treatment method that has recently gained interest due to its confirmed applications in the food industry. In the present study, EW showed a high bactericidal effect against bacteria, yeasts and moulds as compared to sodium hypochlorite. EW treatment induced plant response diminished pathogen infection at industrial scale and showed an impact on the fungal composition. The overall desirable effects described here for electrolyzed water treatments on the postharvest sanitization of cv. 'Granny Smith' apple fruits are encouraging for the agriculture and horticulture industry, especially in consideration of the drive to move away from chemical-based sanitizers. We suggest that the apple fruit is protected by either EW treatment or the inherent microbiome, however presumably. Recent research reports also show the combination of different physical and chemical treatment methods with electrolyzed water to get the maximum antimicrobial effect without compromising the quality of the food products. Moreover, electrolyzed water is a promising and sustainable future strategy to prevent postharvest decay of fresh and stored produce.

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CHAPTER 6

General Summary and Conclusion

The aim of this study was to determine the impact of electrolyzed water (EW) treatments on the pathological and physiological responses, physicochemical, phytonutrients, microbial inactivation and overall quality of 'Granny Smith' apples during storage. The objectives in the study were:

1. To conduct an extensive literature review on the use of electrolysed water treatments (Chapter 2).
2. To determine the curative efficacy of SAI-EW and AEW treatments against *B. cinerea* and *P. expansum* in 'Granny Smith' apples during storage (Chapter 3).
3. To investigate the impact of alkaline electrolysed water treatment (AIEW) on phyto-compounds, antioxidant activity, physical and biochemical quality attributes and natural microbial load of 'Granny Smith' apples (Chapter 4).
4. To characterize the change in the dynamics of culturable microbial community on the surface of 'Granny Smith' apples treated with AIEW (Chapter 5).

The first objective was successfully accomplished where an extensive review on the use of electrolysed water treatment on apples was conducted. The review looked at the nutritional benefits of apple fruits, their physicochemical parameters, post-harvest physiological and pathological disorders, as well as the use of thermal and non-thermal postharvest treatments for the disinfection of apple fruit. The review further described electrolysed water as an emerging antimicrobial treatment method that has recently gained interest in the fruit industry based on previous literature.

In experiment 1 of the second objective, the electrolyzed water treatments (SAIEW) 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 concentrations 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*. Using higher concentrations of EW above 300 mg L^{-1} showed adverse effects on lenticels. In experiment 2 of the second objective, both SAI-EW and AEW had significant curative efficacy against the growth of *B. cinerea* and *P. expansum*. However, based on the outcomes of this study, dipping

‘Granny Smith’ apples in SAI-EW and AEW at a concentration of 100 mg L^{-1} was not effective. The curative efficacy of the electrolyzed water treatments was AEW> SAI-EW. In addition, the findings demonstrated the potential of both SAI-EW and AEW electrolyzed water as an alternative fruit decay management strategy.

Furthermore, the third objective was also successfully accomplished. The study was able to demonstrate that alkaline electrolysed water (AIEW) 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. Additionally, compared to the control this application was effective in enhancing and maintaining total polyphenol, flavonol, flavanol, and antioxidant capacity of ‘Granny Smith’ apples. However, treatment with AIEW was not successful in inhibiting or suppressing the symptoms of superficial scald. Further, when compared with the control and chlorinated water treatment, the AIEW treatment ensured a high reduction in natural microbial load. These results indicated the importance of selecting appropriate dipping duration and highlighted the need to better understand the impact of electrolysed water treatments on quality attributes of ‘Granny Smith’ apples.

Finally, the fourth objective was successfully accomplished by characterizing the change in the dynamics of culturable microbial community on the surface of ‘Granny Smith’ apples treated with AIEW. The results of this study showed a high bactericidal effect against bacteria, yeasts and moulds as compared to sodium hypochlorite. AIEW treatment induced plant response diminished pathogen infection at industrial scale and showed an impact on the fungal composition. It was further suggested that the apple fruit was protected by either the AIEW treatment or the inherent microbiome, however presumably.

In future the following could be of interest:

- The investigation of the concerns related to the use of electrolyzed water in fruit and vegetables.
- The quality of vegetables and fruits after the treatment of electrolyzed water should also be investigated.
- Another necessity in the processing of the food products is the on-site and inexpensive production of the chemical sanitizers. The chemical sanitizer currently being used does not show such characteristics. Therefore, the commercialization of electrolyzed water generation systems can be expected in the near future.

This research has therefore provided South African growers and exporters and indeed the global fruit industry, with an alternative technology to chemical fungicides and sodium hypochlorite for controlling natural spoilage microorganisms and pathological disorders on 'Granny Smith' apples in the fresh apple pack-house. Electrolysed water treatments (EW) offer an alternative for long term storage of 'Granny Smith' apples. EW showed a high bactericidal effect against bacteria, yeasts and moulds as compared to sodium hypochlorite. EW treatment induced plant response diminished pathogen infection at industrial scale and showed an impact on the fungal composition. In addition the EW treatments had desirable effects on the total polyphenols, flavonoids and antioxidant activity of the apple. Apples treated with EW maintained low pH, titratable acidity (TA) and total soluble solids (TSS) compared to other treatments ($p > 0.05$). A major advantage of EW found in this study is that it requires no installation of software, however, this treatment did not offer 100% control of superficial scald on 'Granny Smith' apples, hence the need of possibly combining the treatment with lower dosages of 1-MCP. The overall desirable effects described here for electrolyzed water treatments on the postharvest sanitization of 'Granny Smith' apple fruit are encouraging for the agriculture and horticulture industry, especially in consideration of the drive to move away from chemical-based sanitizers.

Appendix A: Ethics exemption letter

Cape Peninsula University of Technology

Statement of Permission Data/Site permission is required for this study.

Reference no.	213293919/10/2020
Surname & name	Nyamende, N.E.
Student Number	213293919
Degree	Master of Food Science and Technology
Title	Impact of non-thermal postharvest treatments on the physiological responses, phytonutrients and overall quality of apples.
Supervisor(s)	DR ZANEPHYN KEYSER
FRC Signature	
Date	2020 Oct 04

Cape Peninsula University of Technology

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Ethics Exemption Letter **Reference no: 213293919/10/2020**

Office of the Chairperson **Research Ethics Committee**	**Faculty of Applied Sciences**

On 02 October 2020, the Faculty Research Ethics Committee of the Faculty of Applied Sciences determined that the research activities related to a project to be undertaken by Nyamende, N.E. for a degree (Master of Food Science and Technology) at the Cape Peninsula University of Technology does not require ethics clearance. The ethics exemption for the project is approved.

Title of project:	Impact of non-thermal postharvest treatments on the physiological responses, phytonutrients and overall quality of apples.

Comments (Add any further comments deemed necessary, e.g. permission required)

1. Human subjects are not included in the proposed study. 2.

This permission is granted for the duration of the study.

3. Research activities are restricted to those detailed in the research proposal.

4. The research team must comply with conditions outlined in AppSci/ASFREC/2015/1.1 v1, CODE OF ETHICS, ETHICAL VALUES AND GUIDELINES FOR RESEARCHERS.

 **Signed: Chairperson: Faculty Research Ethics Committee**	02/10/2020 **Date**