DEVELOPMENT OF A SMALL-SCALE IN-FIELD INTEGRATED POSTHARVEST CITRUS TREATMENT UNIT

A Kassim

Submitted in partial fulfilment of the academic requirements of
Doctor of Philosophy in Engineering

Agricultural Engineering
School of Engineering
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Pietermaritzburg
South Africa

December 2016

Supervisor: Professor TS Workneh
Co-Supervisor: Professor MD Laing

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

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TS Workneh

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DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this dissertation (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1:
Kassim, A, Workneh, TS, Laing, MD and Basdew, IH. 2016. The effects of different pre-packaging treatments on the quality of kumquat fruit. CYTA-*Journal of Food* 14(4):639-648.

Author contributions:
Alaika Kassim was the main author and responsible for obtaining and reviewing the relevant literature and conducting the necessary experiments. Professor TS Workneh, Professor MD Laing and Doctor IH Basdew provided feedback, guidance and editing.

Submitted 1:
Development and evaluation of a small-scale in-field integrated postharvest citrus treatment unit – Part 1
Journal: International Journal of Food Engineering
Submission number: DGIJFE.2016.0192
Revision number: DGIJFE.2016.0192.R1.

Author contributions:
Alaika Kassim was the main author and responsible for obtaining and reviewing the relevant literature and conducting the necessary experiments. Professor TS Workneh and Professor MD Laing provided feedback, guidance and editing.

Submitted 2:
Treatment Comparison of Kumquat Fruit Using the Postharvest Citrus Treatment Unit for Transport Conditions Simulation – Part 2

Journal: International Journal of Food Engineering

Submission number: DGIJFE.2016.0385

Author contributions:

Alaika Kassim was the main author and responsible for obtaining and reviewing the relevant literature and conducting the necessary experiments. Professor TS Workneh and Professor MD Laing provided feedback, guidance and editing.

Main author: ..... Date: .....

A Kassim

Co-author: ..... Date: .....

TS Workneh

Co-author: ..... Date: .....

MD Laing

Co-author: ..... Date: .....

IH Basdew

ACKNOWLEDGEMENTS

As I compile this document, it has dawned on me that three years' worth of research has come to an end. It was a challenging, and at times almost impossible task, yet wonderful and inspiring. I have not only increased my academic knowledge but have grown as a person. I hope that this document is worth the read and is an indication of the passion and hard work that I have poured into it. However, this was only possible with the help of many others and I would like to express my gratitude and thanks.

I would first want to thank God Almighty, without Whom nothing that ever was, ever will be and is, would be possible and for granting me the opportunity of pursuing a PhD.

I am grateful to my supervisor, Professor Tilahun Seyoum Workneh of the School of Engineering at the University of KwaZulu-Natal, for the continuous support, patience, motivation, confidence and immense knowledge he has granted me for my PhD study and related research. I have not only learnt how to conduct myself professionally but personally as well. He has truly inspired me and I could not imagine having a better supervisor and mentor for my PhD study.

I would like to thank my co-supervisor, Professor Mark Delmege Laing, for the insightful discussions, constructive criticism, ideas and support. It would not have been possible for me to take this work to completion without your guidance and encouragement.

I would like to express my appreciation and gratitude to my beloved family, whose constant support, encouragement and love I have relied upon throughout my life. I am especially thankful to my mum, Firoza Kassim, and my dad Rasheed Kassim, whose unflinching courage, strength, conviction, faith and love will be the fuel to keep me going always. Words cannot suffice to express how truly appreciative I am.

A special thanks to Mr Asif Satar who has supported me during the most crucial times of my studies and assisted me with my research despite the various challenges at hand. He always encouraged me not to give up and never failed to make me smile despite the situation.

Thank you to Mr Mbuso Cele and his proficient team from the Mechanical Instrument Workshop at the University of KwaZulu-Natal for assisting in the construction process.

Thank you to Dr Iona Basdew for the enlightening discussions, motivation and efficient editing of my chapters.

I am indebted to the University of KwaZulu-Natal, which provided the necessary financial aid, infrastructure and resources required for me to conduct my research to the best of my ability.

I am thankful to the National Research Foundation (NRF) for the financial support in conducting my research and being able to attend conferences to exhibit my work.

I am grateful to the Cannon Collins Educational Trust for their financial support and scholarly assistance in ensuring that my research was progressing as I had intended.

I am thankful to Mr Stephen Beghin and Mr Hendrik Steyn of Premier Fruit Exports (Pty) Ltd, Hillcrest, South Africa and Tommie Rautenbach of Rooister Boerdery, Limpopo Province, South Africa in providing me with the necessary information required to conduct this research. I am also thankful to them for the high quality fruit samples, without which my research would have not been possible and for the keen interest in this research.

Thanks to Mr Don McDougal and Mr Philip Nel of Radical Waters (Pty) Ltd, Johannesburg, South Africa, for supplying the anolyte water, which was one of the key treatments of this study.

Thanks to Plant Health Products (Pty) Ltd for supplying the B13 biocontrol agent.

A sincere thanks to the following staff of the Agricultural Engineering Department at the University of KwaZulu-Natal, whose support and efforts in assisting me will always be remembered:

- Professor Jeff Smithers.
- Doctor Aidan Senzanje.

- Mrs Natasha Moneyvalu.

I would like to thank the following persons for their academic assistance in providing me with the necessary laboratory space and equipment:

- Mrs Elsie Correia, Senior Technician from Dietetics and Human Nutrition.
- Mr Richard Burgdorf, Senior Technician from Plant Pathlogy.
- Mr Vivek Naiken, Senior Technician from the Center for Water Resources Research.

Lastly, I would like to thank my fellow PhD candidates, Ms Nomali Ngobese and Mr Jean N Namugize, from the Agricultural Engineering and Hydrology Departments for the insightful discussions, encouragement and many laughs. I wish them all of the best in their future endeavours.

ABSTRACT

Once harvested, kumquat fruit continue to respire, which is further exacerbated by elevated temperatures in the field and during transport to packhouses. This results in the proliferation of pathogens, which is detrimental to the postharvest fruit quality and, consequentially, results in a decrease in the fruit shelf life. Bottlenecks in South African packhouses were identified as a challenge due to the large quantity of fruit that need to be processed. The aim of this study was to develop an integrated postharvest citrus treatment unit (IPCTU) used on kumquat fruit. The innovation of this unit is that it condenses the processes of a packhouse into six mobile treatment zones. Additionally, the combined treatment of anolyte water (disinfection), hot water (curative) and B13 (preventative), improved the physical, chemical, microbiological and subjective quality of kumquat fruit and resulted in a 7-day shelf life extension. The IPCTU was constructed from 0.9 mm thick grade 304 stainless steel (food grade) with six treatments zones: (1) primary rinsing, (2) secondary rinsing, (3) anolyte water, (4) hot water, (5) surface moisture removal, and (6) a yeast biocontrol agent (B13). An energy analysis revealed that 4.13 kW and 2.08 kW of electricity was consumed by the hot water tank (HWT) and biocontrol tank (BT) as these tanks required heating. The thermal efficiencies of the HWT and BT were 72% and 87%, respectively. The total carbon ratio for the IPCTU prototype was 0.46 kg $CO_2.day^{-1}$ with a payback period of 0.91 years. *Penicillium digitatum*-inoculated fruit treated with anolyte water × 30s + HWT × 20s × 53°C + B13 exhibited the best quality. These fruit demonstrated low PWL values (37.87%), were firmer (6.20 N), high MC (58.3%), low TSS (11.2 °Brix) by Day 21 and a low CC (5.6 log $CFU.g^{-1}$) immediately after treatment. Anolyte water × 30s + HWT × 30s × 53°C + B13 resulted in fruit possessing the best quality for *P. italicum*-inoculated fruit in terms of low PWL values (27.72%), reduced colour change (70.92°), low MC (62.2%), low TSS (10.7 °Brix) on Day 21 and a low CC (5.5 log $CFU.g^{-1}$) and FC (5.7 log $CFU.g^{-1}$) immediately after treatment. Visible mould formation was observed only on control samples on Day 14. Due to *P. digitatum* being a more prevalent pathogen infecting citrus fruit, it can be recommended that anolyte water × 30s + HWT × 20s × 53°C + B13 be used in conjunction with the IPCTU to improve kumquat fruit quality.

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1. INTRODUCTION

The aesthetic appeal of citrus fruit has a significant effect on the consumer's decision to purchase (Blasco *et al*., 2009). However, the aesthetics and nutritional characteristics of citrus are negatively affected by pathogenic disorders and postharvest handling. Unsuitable fruit handling leads to hastened physiological deterioration, which can manifest in the proliferation of microbiological activity, and accelerated ripening and decay. This can have further market related consequences, resulting in reduced income generation by farmers and a negative perception of importers toward South African citrus fruit.

Kumquat (*Fortunella* spp.) is the smallest of citrus fruit, and was native to China (Choi, 2005; Ladaniya, 2008). Despite this fruit not being a major export crop relative to the more popular citrus cultivars such as orange and grapefruit, there exists an export demand for South African grown kumquats to the European Union and United Kingdom (Beghin, 2014; Department of Agriculture, Forestry and Fisheries, 2014; 2015). Therefore, research is required to improve the manner in which these fruit are handled after harvest to maintain the quality, limit decay and increase the shelf life during storage and transport.

Many studies have identified *Penicillium digitatum* and *P. italicum* to be the most severe postharvest fungal pathogens affecting citrus, including kumquat fruit (Holmes and Eckert, 1999; Altieri *et al*., 2013; Youssef *et al*., 2014). Fungicides have commonly been used to address these problems. However, more environmentally friendly treatments are being sought due to the development of fungal resistance to fungicides, and the growing public demand for safer foods (Ben-Yehoshua *et al*., 2005; Zhang, 2007). Some of these environmentally friendly treatments include hot water, biocontrol agents and anolyte water (Ben-Yehoshua *et al*., 2005; Workneh *et al*., 2011). Therefore, more emphasis needs to be placed on research within the postharvest citrus industry to maximise the potential benefits of improved fruit quality and income via the application of effective pre-packaging treatment techniques.

Exposure of citrus fruit to field heat and ambient conditions during transport from the orchard to the packhouse exacerbates the deterioration process by further increasing fruit

temperature, promoting microbial proliferation (Sullivan *et al*., 1996; Brosnan and Sun, 2001). The use of pre-packaging treatments, such as hot water, surface coatings, ultra-violet irradiation, chlorinated water, biocontrol agents, and carbonate and bicarbonate salts were found to be beneficial in maintaining the postharvest quality of citrus fruit (Porat *et al*., 2000; Njombolwana *et al*., 2013; Youssef *et al*., 2014). Heat treatments have been found to induce fruit tolerance against cold injury and pathogens due to the development of heat shock proteins (Ben-Yehoshua *et al*., 2005). The application of surface coatings or waxes promotes the aesthetic appeal of the fruit and reduces the loss of moisture, thereby extending the fruit shelf life (Johnston and Banks, 1998). Ultra-violet irradiation reduces decay in citrus fruit due to its germicidal effect and its ability to induce the fruit's tolerance to decay (Ben-Yehoshua *et al*., 2005). Treatment of citrus with carbonate and bicarbonate salts can delay postharvest decay by activating the fruit's defence mechanism (Youssef *et al*., 2014). Similarly, the use of chlorine (hypochlorite) as a disinfectant has also extended the citrus fruit shelf life and is widely used in the fruit industry (Workneh *et al*., 2003; Beghin, 2014). Biocontrol agents have been used as an alternative to synthetic fungicides to alleviate postharvest decay (Droby *et al*., 2009; Abraham *et al*., 2010). Anolyte water has demonstrated strong germicidal and disinfecting characteristics when applied to tangerine (Whangchai *et al*., 2010). These pre-packaging treatments have been used with success as individual treatments but more so, the combined effect of a number of these pre-packaging treatments have been beneficial in extending the shelf life of citrus (Korf *et al*., 2001; Obagwu and Korsten, 2003; Ben-Yehoshua *et al*., 2005; Hong *et al*., 2014; Moscoso-Ramirez and Palou, 2014). However, limited research has evaluated the effects of integrating environmentally friendly treatments on kumquat fruit.

The use of equipment to perform postharvest operations at the packhouse and during harvesting is an existing practice in the citrus industry (Dodd *et al*., 2008). Many advances have been made in the use of brushes and nozzles to rinse and apply treatments, such as waxes, hot water, fungicides and hypochlorite to citrus fruit (Fallik *et al*., 1999; Fallik, 2004; Ladaniya, 2008). However, much of the postharvest processing is confined to packhouses, where bottlenecks are likely due to the large quantity of produce being processed (Ortmann *et al*., 2006). Furthermore, the logistics involved can result in delays in conveying the produce from the field to the packhouse. This consequentially delays disease control treatments, promoting deterioration, decay and loss of fruit (Camelo,

2004). One approach to addressing this deficiency is the implementation of a pre-packaging treatment unit capable of operating at the orchard. Pre-packaging in the orchard can reduce the time lapse from harvest to treatment, which will ultimately improve the fruit quality and shelf life (Sullivan *et al*., 1996). The approach of combining a number of pre-packaging treatments to treat kumquat fruit has not previously been documented in literature. More importantly, the method of treatment application by using a mobile unit capable of operation in the orchard provides an innovative practice, which can be applied to other horticultural commodities, thus revolutionising the postharvest treatment sector. It is essential that these techniques be developed to maintain the fruit quality from the point of harvest to the final market destination.

Attention to small-scale citrus farmers in South Africa has been neglected due to the industry being primarily export-based and reliant on more established commercial farmers. However, the development of small-scale farmers is crucial for expanding current markets, food security and job creation. Improving the current postharvest technologies will assist small-scale farmers. It is hopeful that this unit may successfully be adopted by small-scale farmers to improve the quality of fruit marketed by this sector.

The overall aim of this study was to develop an in-field, integrated postharvest citrus treatment unit (IPCTU) and to evaluate the effects of the pre-packaging treatments applied using the in-field experimental unit on the postharvest quality of kumquat fruit (*F. margarita*).

The specific objectives formulated for this study were to:

1. Evaluate the effects of various individual and integrated pre-packaging treatments on the physical, chemical, microbiological and subjective quality of kumquat fruit.
2. Develop a unit with multiple pre-packaging treatment zones: (1) rinsing; (2) disinfection; (3) hot water; (4) surface moisture removal; and (5) a biocontrol agent application.
3. Evaluate the overall efficiency of the pre-packaging treatment unit.
4. Identify the most effective treatment of kumquat fruit that can be implemented by small-scale farmers using the integrated postharvest citrus treatment unit.

The research questions that arose were:

1. What suitable and environmentally friendly technologies can be applied to kumquat fruit?
2. Can suitable pre-packaging treatments be easily sourced or produced and applied?
3. Can suitable pre-packaging treatments be used in an integrated manner? If so what are the effects on the postharvest quality of kumquat fruit – beneficial or harmful?
4. What is the most effective combination of pre-packaging treatments on the kumquat fruit quality?
5. Can a mobile unit be designed to incorporate suitable pre-packaging treatments to be used on site at the orchard?
6. Can a mobile pre-packaging treatment unit be adopted on a farm scale?
7. What are the benefits of an on-farm integrated pre-packaging treatment unit?
8. How can the current citrus supply chain be optimised?

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2. A REVIEW OF LITERATURE ON THE POSTHARVEST CHARACTERISTICS, TREATMENTS AND HANDLING OF CITRUS FRUIT

2.1 Abstract

Access by small-scale kumquat farmers to the international export market is required to allow for greater income generation and for the development of the South African citrus industry. Once harvested, fruit continue to respire, which is further exacerbated by elevated temperatures in the field and during transport to packhouses. This results in the proliferation of pathogens, which is detrimental to the postharvest fruit quality and, consequentially, results in a decrease in the fruit shelf life. Bottlenecks in South African packhouses have been identified as a challenge due to the large quantity of fruit that need to be processed. Limited research on postharvest quality issues of kumquat, particularly in South Africa, is available in literature. This warrants the need for postharvest research to be undertaken on kumquat fruit.

The aim of this chapter is to highlight the pre-packaging treatments of citrus fruit and the equipment involved in such treatments. Hot water, surface coatings, ultra-violet irradiation, chlorine (hypochlorous), salt treatments and microbial antagonists have been beneficial in maintaining the citrus quality and reducing the prevalence of postharvest decay. Environmentally friendly anolyte water has also proven to be a favourable postharvest treatment of carrots and tomatoes. Integrated treatments, such as hot water treatments and chlorine disinfection, have been successfully used in the global citrus industry. The use of integrated pre-packaging treatments improved the quality and shelf life of citrus, compared to individual treatments. An effective combination of pre-packaging treatments should include: (1) disinfectant; (2) curative and (3) preventative treatments to control pre- and postharvest pathogens. The equipment and machinery responsible for treating citrus fruit are predominantly situated in packhouses, which require that fruit be transported a distance after harvest. This contributes to quality degradation due to pathogenic infections such as *Penicillium* spp. Treating fruit directly after harvest in the orchard, compared to at a packhouse, introduces an innovative method of addressing the current challenges in the citrus industry. Research is required to improve

and optimize the postharvest handling technologies for citrus fruit, specifically kumquats in South Africa.

2.2 Introduction

The aim of the literature review was to identify suitable pre-packaging treatments, technologies and equipment to improve the quality of citrus fruit, specifically kumquat fruit, with a focus on postharvest trends in the citrus industry (Section 2.6). An introduction to kumquats and the harvesting techniques are presented in Section 2.3. An outline of the physical, chemical, and microbiological quality parameters associated with evaluating the quality of citrus fruit are provided in Section 2.4. Subsequently, the effects of different pre-packaging treatments applied to citrus fruit are discussed (Section 2.5). The citrus supply chain and markets are explained in Section 2.7 followed by a discussion and conclusion in Section 2.8.

2.3 Harvesting and Disorders Affecting Kumquat Quality

This section discusses the effect of the harvesting technique, and pathological and physiological disorders, on the quality of kumquat fruit.

2.3.1 Introduction to kumquat fruit

Kumquat (*Fortunella* spp.) belonging to the family Rutaceae, to which citrus belongs, is the smallest of citrus fruit and is believed to have originated in China (Hall, 1986; Choi, 2005; Ladaniya, 2008; Schirra *et al*., 2008; Peng *et al*., 2013). The two most common kumquat varieties are Nagami (*F. margarita*) and Marumi (*F. japonica*) (Young, 1986; Saunt, 1990) as indicated in Figures 2.1 (a) and 2.1 (b), respectively. Nagami are oval with a slightly wider stylar-end of approximately 39 mm in length and weigh around 14 g (Jaliliantabar *et al*., 2013). Marumi are more rounded to slightly oval, and are smaller than Nagami, with a mean weight of 12 g. The juice content of kumquats is approximately 15-17% °Brix, 4-5% acid and 50-55 mg ascorbic acid per 100 mL, but this may vary, depending on the variety and growing regions (Ladaniya, 2008). The optimum storage temperatures for kumquats range from 4.5°C to 11.0°C (Beghin, 2014c; Department of Agriculture, Forestry and Fisheries, 2015b). Cultivation of kumquats in South Africa,

aimed primarily at the export market, is concentrated in the Letsitele region, just outside Tzaneen and Levubu near the Kruger National Park.

(a)

(b)

Figure 2.1 Kumquat fruit (a) Nagami (*Fortunella margarita*) and (b) Marumi (*Fortunella japonica*) (Saunt, 1990)

Citrus fruit can be classified as being non-climacteric, with low rates of respiration and ethylene evolution during the ripening stage (Porat *et al*., 2004; Ladaniya, 2008; Li *et al*., 2008). This allows for extended storage periods of six to eight weeks (variety dependant) (Porat *et al*., 2004; Li *et al*., 2008). However, Chalutz *et al*. (1989); cited by Schirra *et al*. (2011), noted that kumquat fruit are susceptible to rapid decay due to infection caused by *Penicillium* pathogens. Similarly, Li *et al*. (2008) observed that after harvest, under ambient conditions, kumquats can lose excessive moisture and become wrinkly. Therefore, effective postharvest handling procedures of kumquat fruit, such as pre-packaging treatments need to be developed to alleviate these detrimental effects.

2.3.2 Impact of harvesting techniques

Grierson and Ben-Yehoshua (1986) identified harvesting as being the single most critical factor influencing fruit quality during storage and transportation. The characteristics of kumquat fruit are similar to those of other citrus in that they are unable to ripen once harvested unripe and, therefore, they should be picked when fully ripe (Kader, 1999; Ladaniya, 2008). The onset of postharvest decay in citrus fruit is largely dependent on cultural practices, such as the method and time of harvest, and pre- and postharvest factors (D'hallewin *et al*., 1999; Beghin, 2014a). Once harvested, the fruit become more susceptible to microbiological infections as it is detached from the plant (D'hallewin *et*

al., 1999). McGuire and Reeder (1992) found that late and early season grapefruit succumbed to greater damage (scalding) when exposed to air heated to 46°C, 48°C, and 50°C for three, five or seven hours, compared to mid-season fruit after harvest. This could be attributed to early season fruit having immature skins and late season fruit already beginning to senesce. Dessert lemons and blood oranges are most susceptible to chilling injury when harvested early in the season (Houck *et al*., 1990; Schirra *et al*., 1997).

Currently citrus harvesting is done manually by hand as this method results in the least damage to the fruit, which minimises the risk of early decay and inferior postharvest quality (Schueller *et al*., 1999; Sanders, 2005). Mechanical harvesting in the citrus industry has not been a prominent feature because it lacks the flexibility and fruit selection ability of manual harvesting (Sanders, 2005). However, more automated systems employing the desired selection criteria for individual citrus fruit have been developed by Jimenez *et al*. (2000). Harvesting of kumquats in South Africa takes place from May to October, when fruit are picked continuously because both flowers and fruit appear on trees at the same time (Beghin, 2014a; 2014b). Kumquat fruit stems are clipped rather than snapped because the latter may induce fruit injury. Fruit that are yellow to orange are ready to be picked (Beghin, 2014b). A small portion of the pedicel is still attached to the kumquat because it cannot easily be removed without injuring the fruit. However, it is this portion of the stem that regularly causes injury to adjacent fruit in containers, which hastens fruit deterioration (Beghin, 2014a; Laing, 2014a). This problem requires research to be conducted to optimise kumquat harvesting. However, this was not included in the scope of this study. Once harvested, each worker places the kumquats into bags, which are then weighed and transferred into 18-20 kg lug boxes (Beghin, 2014a). The pickers also play a pivotal role by practicing hygienic methods of harvesting to prevent *Escherichia coli* contamination of fruit (Laing, 2014c). This can be addressed by providing pickers with portable toilets, a suitable disinfectant and water. Pickers should also avoid picking fruit from the ground to minimise infection as the fruit may have been damaged when it fell to the ground.

2.3.3 Pathological and physiological disorders

Harvested commodities need to be cleaned of any dirt, debris, insects and synthetic chemicals prior to packaging to extend the shelf life and for the consumer (Fallik, 2004).

Porat *et al*. (2004) identified two factors that limit the postharvest shelf life of citrus: (1) pathological breakdown and (2) physiological breakdown. Pathological decay is caused by fungi or bacteria, whereas physiological breakdown is initially as a result of biotic factors, which weaken the fruit and affect its ability to ripen properly (Boyette *et al*., 1993, Ladaniya, 2008). Droby *et al*. (1998), Ladaniya (2008), Schirra *et al*. (2011), Gomez-Sanchis *et al*. (2012), Altieri *et al*. (2013), and Youssef *et al*. (2014) have all identified *Penicillium digitatum* and *P. italicum* as the most severe postharvest pathological infections affecting citrus fruit. Citrus fruit under ambient conditions are mainly susceptible to green mould caused by *P. digitatum* Sacc., which may result in 60-80% fruit decay while blue mould is as a result of *P. italicum* Wehmer exhibited by fruit stored under cold storage (Figure 2.2 (a)). Strict postharvest and packhouse sanitation is required to restrict fruit losses as a result of blue and green moulds (Ladaniya, 2008).

Citrus black spot (CBS) has recently contaminated South African citrus exports to the European Union (EU) after the disease was detected in some of the shipments, as explained by Mokomele (2013). As of 29 November 2013, the Standing Committee on Plant Health stated that only citrus from areas free of CBS in South Africa could be exported to the EU for that particular season (Mokomele, 2013). However, according to Yanowa *et al*. (2013), the CLIMEX model, which simulates an organism's response to a particular climate worldwide, showed that CBS poses an exceedingly low risk to the citrus producing regions in Europe. Figure 2.2 (b) illustrates freckle spot caused by CBS.

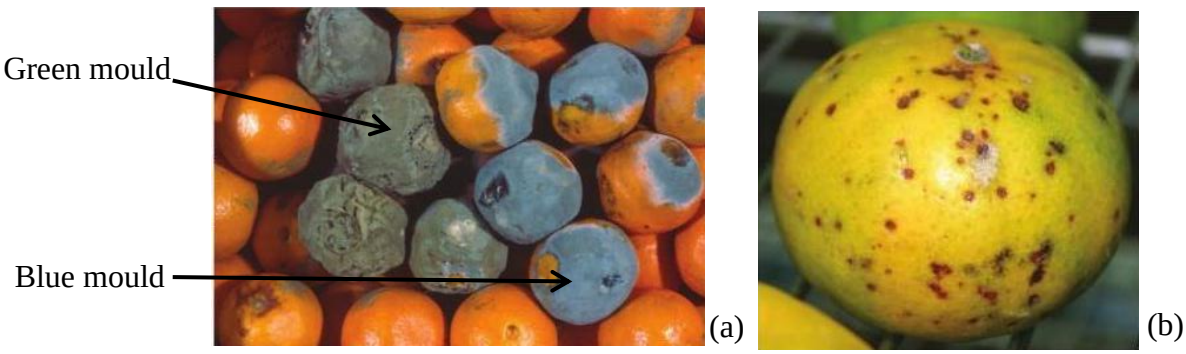

Figure 2.2 (a) Green mould caused by *Penicillium digitatutm* and blue mould caused by *Penicillium italicum* and (b) freckle spot caused by citrus black spot (Cooke *et al*., 2009)

Sour rot has also been described as a postharvest disease resulting in significant losses in citrus fruit (Merciera and Smilanick, 2005; Talibi *et al*., 2012). Losses are particularly greater during the wet season and fruit degreening (Talibi *et al*., 2012). Sour rot requires open wounds on the citrus fruit for entry and proliferation (Ladaniya, 2008; Talibi *et al*., 2012). Stem-end rind breakdown is classified as a physiological disorder, which can be attributed to an imbalance in potassium and nitrogen. However, its development is dependent on the handling procedures between picking and packaging (Grierson, 1986). This disorder results in the collapse and darkening of the epidermal tissue around the stem-end of the fruit. The loss in fruit moisture promotes stem-end rot (Grierson, 1986; Wardowski, 1988b; Ritenour *et al*., 2004). Grierson (1986) recommends that fruit be transported immediately after harvest to the packhouse and maintained at high relative humidity (>90%). Furthermore, during pre-treatment, brush speeds should not exceed 100 rpm (Grierson, 1986).

Table 9.1 in Appendix A lists some of the pathological and physiological diseases and disorders exhibited by citrus fruit. The scope of this study focused primarily on improving and maintaining the quality of citrus fruit, particularly kumquats, by reducing decay due to *P. digitatum* and *P. italicum*.

2.4 Postharvest Quality of Citrus

Ladaniya (2008) defines fruit quality as the combination of fruit attributes that have a significant influence in determining consumer acceptance and willingness to purchase. It is imperative that citrus fruit attain both the internal and external quality standards at harvest. This section reviews the physical, chemical, and microbiological quality of citrus fruit.

2.4.1 Physical quality parameters

The physical properties are associated with the appearance, aesthetics and response of the fruit to certain external stimuli such as forces (tensile and compressive) during loading and stacking and light exposure during postharvest handling (Ladaniya, 2008). The physical properties of citrus fruit discussed in this section include skin colour, weight loss, and firmness.

2.4.1.1 Skin colour

The colour perception of citrus fruit is an important factor in determining a customer's willingness to purchase (Olmo *et al*., 2000; Singh and Reddy, 2006). Colour measurement can be carried out either subjectively or objectively, as in the case of firmness (Section 2.4.1.3). Subjective colour measurement is determined visually by eye. Ladaniya (2008) describes a colour scale system, which divides samples into different colour categories of deep green, light green, yellowish-green, greenish-yellow, yellowish-orange, orange, and deep orange. This scale may vary depending on the citrus cultivar. Objective colour measurements make use of calibrated equipment such as colour meters (Pathare *et al*., 2013). The parameters associated with colour include L (lightness or brightness), a* (redness or greenness), b* (yellowness or blueness), hue and chroma (Pathare *et al*., 2013).

The colour change in citrus fruit can be attributed to the conversion of chloroplasts to chromoplasts, resulting in a loss of chlorophyll and the synthesis of carotenoids (Olmo *et al*., 2000; Ortiz, 2002; Singh and Reddy, 2006; Iglesias *et al*., 2007). Ortiz (2002) attributed the yellow colour in citrus to carotenes and xanthophylls, and the reddish colour to anthocyanin. The application of exogenous ethylene during the process of degreening has been found to accelerate the development of carotenoids in citrus fruit and to improve colour development (Stewart and Wheaton, 1971; Rodrigo and Zacarias, 2007). Rodov *et al*. (2000) found that hot water brushing of citrus fruit at 60°C delayed the colour change from green to yellow by two weeks. This could be due to the production of heat shock proteins, which inhibit senescence. Smilanick *et al*. (2006) found that the postharvest application of sodium bicarbonate, either alone or in combination with thiabendazole fungicide, resulted in a detectable but minor delay in the colour change during the process of degreening.

2.4.1.2 Weight loss

Weight loss is an important factor in citrus fruit deterioration and is often accompanied by a decrease in firmness (Porat *et al*., 1999). Citrus fruit have a high moisture content in both the pulp and peel (Chien *et al*., 2007; Ghanema *et al*., 2012). The loss of moisture via transpiration and respiration occur rapidly after harvest, promoting fruit decay (Purvis,

1983; Chalutz *et al*., 1989). Much of the moisture is lost from the peel tissue, leading to shrivelling, shrinkage, softening and deformation, affecting the fruit appearance. The weight loss in heat-treated mandarins was significantly lower than in ultra-violet (UV) irradiated fruit at 4.10 g and 5.34 g, respectively (D'hallewin *et al*., 1994). The use of waxes reduces the loss in moisture in many horticultural crops (Hall, 1981; Hagenmaier and Baker, 1994; Chien *et al*., 2007). However, over-waxing can lead to off-flavours and odours (Hall, 1981; Purvis, 1983). Cohen *et al*. (1990) found that the use of water-based polyethylene waxes on Murcott tangerines reduced the weight loss but also led to an inferior taste, compared to un-waxed fruit. According to Ben-Yehoshua *et al*. (1985), waxes block the stomatal pores, hindering gas exchange to a greater extent than moisture. It was further observed that individually wrapping oranges and grapefruit in high density polyethylene films reduced moisture loss by 90% without detrimentally restricting gas exchange, compared to waxing. Kumquat fruit dipped in hot water (53°C for 120 seconds) displayed a lower weight loss, compared to control samples (Rodov *et al*., 1995). Heat treatments have a profound effect in reducing weight loss of citrus fruit. Fruit moisture loss, due to the vapour pressure deficit at the time between harvest and packing, leads to an increase in the incidence of pitting (Citrus Growers' Association, 2013).

2.4.1.3 Firmness

In citrus fruit, the firmness can be defined as the resistance to puncture, which is a mechanical properties of the fruit. Fruit firmness is often used as a criterion to determine the effects of storage and shelf life (Singh and Reddy, 2006). Firmness tests include puncture resistance, compression, creep, impact and sonic tests (Abbott, 1999). Instruments commonly used to measure citrus firmness include texture analysers, and handheld penetrometers, which constitutes objective methods. Subjective techniques include hand-feel due to the viscous component of citrus fruit (Abbott, 1999; Ladaniya, 2008). The peel of the citrus fruit is composed of the flavedo (exterior coloured portion) and the albedo (white inner portion), which resists exerted forces. Beneath the peel are segments composed of juice sacs or juice vesicles, which offer minimal resistance to applied forces. With an increasing moisture loss, the peel becomes tough and leathery. Heat-treated mandarins resulted in superior fruit firmness, compared to the control and UV treated samples (D'hallewin *et al*., 1994). Similar results were obtained by Rodov *et al*. (2000), where hot water dipping (52°C for 120 seconds) and hot water brushing (60°C)

resulted in firmer fruit than non-treated samples. Citrus fruit coated with chitosan wax and those treated with thiabendazole fungicide were firmer, compared to control samples, after 56 days of storage at 15°C (Chien *et al*., 2007).

Citrus fruit firmness primarily depends on cell turgidity, which is associated with the moisture content. Rodov *et al*. (1995; 2000) observed that heat treatments assist in redistributing the natural epicuticular wax, which seals microscopic cracks, preventing the escape of moisture, promoting cell turgidity and firmer fruit. Heat treatments may also improve fruit firmness by inhibiting enzyme activity involved in fruit softening or by cell wall strengthening (lignification).

2.4.2 Chemical quality parameters

Chemical properties primarily provide information regarding the taste, flavour, aroma and nutritive value of horticultural commodities. The chemical properties discussed in this section are total titratable acid, total soluble solids, and the maturity index.

2.4.2.1 Total titratable acid

Organic acids play a major role in the organoleptic characteristics of citrus fruit. Citric acid accounts for approximately 80-95% of the total titratable acids (TTA) in citrus fruit (Ladaniya, 2008). Generally, there is a decrease in the TTA of citrus fruit during ripening, depending on the cultivar (Olmo *et al*., 2000; Sadka *et al*., 2000; Ortiz, 2002; Albertini *et al*., 2006; Ladaniya, 2008). This can be attributed to the catabolism of citric acid as well as an increase in the total sugars, resulting in mature fruit having lower acidity (Iglesias *et al*., 2007). Sadka *et al*. (2000) found that a high acid content in mature citrus fruit can reduce the quality and delay harvest. The method commonly used to measure TTA is titration (Lobit *et al*., 2002; Hong *et al*., 2007; Ladaniya, 2008). Other advanced methods make use of magnetic resonance (Abott, 1999). Purvis (1983) found that the acid content in grapefruit and oranges decreased during storage. Similarly, Baldwin *et al*. (1995) observed a decrease in the citric acid of oranges after four weeks of storage. The TTA in fresh cut oranges stored at 4°C was found to decrease from 0.46% to 0.29% over a 13-day storage period (Rocha *et al*., 1995). Hong *et al*. (2007) found that heat-treated mandarins did not display a significant change in the TTA.

2.4.2.2 Total soluble solids

The total soluble solids (TSS) of citrus fruit contribute approximately 10-20% of the fresh weight. About 70-80% of the TSS are carbohydrates (Iglesias *et al*., 2007). Other minor constituents of TSS include organic acids, proteins, lipids and minerals (Olmo *et al*., 2000; Iglesias *et al*., 2007; Ladaniya, 2008). TSS determination is based on the refractive index of the fruit juice using a refractometer. Rodov *et al*. (2000) found a gradual increase in the TSS of citrus fruit during storage. This is due to the loss in moisture resulting in an increase in the solute concentration. D'hallewin *et al*. (1994) found that the TSS in heat-treated (36°C for 72 hours) and UV-treated (24 nm) Avana mandarins were lower than control samples at 7.85, 7.63 and 8.02 °Brix, respectively. Baldwin *et al*. (1995) found that coated oranges had a slightly lower TSS, compared to uncoated fruit stored at 16°C or 21°C; however, this was not significant. Purvis (1983) did not find any significant change in the TSS of waxed oranges and grapefruit. Contrary to these observations, Hong *et al*. (2007) found a decrease in the TSS, which was attributed to consumption of sugars and organic acids for plant metabolism in mandarins during storage.

2.4.2.3 Maturity index

The maturity index can be determined by the ratio of TSS:TTA (D'hallewin *et al*., 1994; Olmo *et al*., 2000; Ortiz, 2002; Iglesias *et al*., 2007). This serves as an indication of the legal maturity of oranges, mandarins, grapefruit, pummelos and their hybrids (Ladaniya, 2008). The maturity index is also used to determine the relative sweetness or sourness of citrus fruit. The maturity index tends to increase due the increase in the soluble solids and the decrease in the organic acids (Olmo *et al*., 2000). Higher ratios generally imply a decrease in the acidity; however, this is dependent on the contributions of both TSS and TTA. The highest maturity index of Avana mandarins was observed for heat treatments at 36°C for 72 hours (16.77), compared to UV treatment (15.48) (D'hallewin *et al*., 1994). The maturity index for an acceptable flavour quality in grapefruit, mandarin and orange were found to be approximately 6+, 8+ and 8+, respectively (Kader, 1999).

2.4.3 Microbiological quality

2.4.3.1 *Penicillium digitatum* and *Penicillium italicum*

Citrus fruit treated by hot water dipping at 52°C for 120 seconds, or thiabendazole wax, or curing at 36°C for 72 hours, all controlled the development of *Penicillium* moulds (Rodov *et al*., 2000). The incidence of citrus decay was also reduced by hot drench brushing treatments at 56 or 60°C. Similar results were obtained for kumquats in which fruit were dipped in water at 52°C for 120 seconds. This effectively reduced decay during four weeks of storage (Rodov *et al*., 2000). Hot water brushing for 20 seconds at 56°C reduced decay development due to *P. digitatum* by 80% (Porat *et al*., 2000). The optimum curing temperature inhibiting *P. digitatum* growth in oranges was found to be at 35°C for 48 hours. However, this resulted in an increase in the occurrence of stem-end rot after two weeks (Zhang and Swingle, 2005). The application of 500-2000 $mg.L^{-1}$ of fludioxonil fungicide reduced the presence of green mould (Zhang, 2007). Ultra-violet-C (UV-C) irradiation has also shown to significantly reduce the incidence of blue and green mould. However, the risk of over dosage may lead to the development of phytotoxins (Palou *et al*., 2008).

Based on the physical, chemical and microbiological quality parameters that have been discussed, the main question that arises is what are the effects of different pre-packaging treatments (individually and combined) on the quality of kumquat? The need to quantify this is required, specifically for kumquat fruit in South Africa, in order to obtain a greater understanding of the postharvest characteristics and behaviour of kumquat fruit that can be applied in the citrus industry.

2.4.3.2 Citrus black spot

Citrus black spot (CBS) caused by *Guignardia citricarpa* (Kiely), attacks the citrus fruit and foliage, resulting in unsuitable fruit for the fresh market (Bonants *et al*., 2003; Yonow *et al*., 2013). Infection occurs via both pynidia and ascospores, which may be present on infected leaves on the orchard floor (Korf *et al*., 2001). CBS has usually been controlled with copper fungicides. However, this leads to darkening of citrus blemishes and an undesirable accumulation of copper in the soil (Schutte *et al*., 1997). Agostini *et al*. (2006)

found that postharvest fungicide treatments alone had minimal effects in reducing CBS symptoms. However, the application of fungicides during fruit growth and storage of harvested fruit at 8°C immediately after harvest was effective in reducing CBS symptoms.

More environmentally friendly methods, such as heat treatments and waxing, have been used with success to alleviate CBS. The application of skin coatings to oranges was found to reduce the onset of CBS, which could be associated with reduced respiration rates (Seberry *et al*., 1967). Seberry *et al*. (1967) recommended that postharvest treatments complement orchard control methods to control CBS. Korf *et al*. (2001) found that conidial germination on CBS-infected fruit was reduced to zero with postharvest treatments of hypochlorite, heat treatments, a chemical mixture, polyethylene wax or all treatments combined. This demonstrated the beneficial application of combined pre-packaging treatments in reducing CBS. Further research is required to determine the feasibility of other combined pre-packaging treatments on citrus.

2.5 Pre-Packaging Treatments

Senescence and decay are natural processes occurring in horticultural commodities and cannot be stopped but merely delayed. This can be achieved by implementing suitable postharvest strategies, such as pre-packaging treatments as outlined in this section.

2.5.1 Heat treatments

Heat treatments have been used to control decay in various fruit, such as avocados (Wu *et al*., 2011; Kassim *et al*., 2013), peppers (Fallik *et al*., 1999; Fallik, 2004; Gonzalez-Aguilar *et al*., 2000) and citrus (Ben-Yehoshua *et al*., 2000; Schirra *et al*., 2008). Heat treatments have the ability to inactivate surface or below surface pathogens, by inducing the fruits' resistance to inhibit pathogen development (Irtwange, 2006; Schirra *et al*., 2011). Heat treatments can therefore, provide a 'curative' treatment (Schirra *et al*., 2000; Irtwange, 2006; Laing, 2014c). Contrastingly, Palou *et al*. (2002) described hot water treatments to be non-curative whose effects are only temporary. However, studies by Kim *et al*. (1991), Ben-Yehoshua *et al*. (1992) and Obagwu and Korsten (2003) demonstrate the curative ability of heat treatments.

The two main protein groups activated by hot water treatments are: (1) heat shock proteins (HSP) and (2) pathogenesis-related proteins (PRP) (Pavoncello *et al*., 2001). HSPs are responsible for inhibiting protein aggregation during high temperatures, thus promoting the fruit's ability to withstand these temperatures. PRPs are thought to contribute to the fruit's defence against a variety of pathogens. Water is the preferred heating medium due to it being more efficient in the heat transfer, compared to air (Fallik, 2004). The benefits associated with heat treatments include reduced chilling injury, increased gloss on the fruit exterior and reduced weight loss, resulting in an increased fruit shelf life (Rodov *et al*., 1995; Irtwange, 2006; Schirra *et al*., 2011). However, excessive heat exposure can result in phytotoxic damage to the fruit (Ben-Yehoshua *et al*., 2000; Irtwange, 2006). This can be avoided by applying higher water temperatures with shorter exposure durations (Fallik, 2004). Contrary to this, McGuire and Reeder (1992) suggested that higher temperatures or extended exposure times should be avoided to prevent early decay. Table 9.2 in Appendix A summarises the effects of different heat treatments on citrus fruit.

Schirra *et al*. (2008) found that kumquat fruit dipped in hot water for 120 seconds at 50°C, then stored at 17°C for 21 days at approximately 80% relative humidity, did not demonstrate significant changes in their nutraceutical and health-related properties. Dipping kumquat fruit in water heated to 53°C for 120 seconds reduced decay during storage (Rodov *et al*., 1995). Similarly, kumquats dipped in hot water for 120 seconds or 30 seconds at 53°C or 56°C resulted in a reduction in incidence of *P. digitatum* and *P. italicum* infections, while exposure to higher temperatures of 59°C and 61°C accelerated the onset of decay (Ben-Yehoshua *et al*., 2000). Hot water treatments do not involve any chemicals, making them environmentally friendly, and are generally easy to apply, which contributes to their industrial appeal. This makes hot water treatments particularly suitable for kumquats due to the manner the fruit is consumed, which includes both the pulp and peel (Ben-Yehoshua, 2000; Schirra *et al*., 2011). Studies conducted on the effect of hot water treatments at 53°C on kumquat fruit have produced favourable results including reduced weight loss, improved appearance and reduced decay (Schirra *et al*., 1995; Ben-Yehoshua *et al*., 2000; Rodov *et al.*, 2000).

2.5.2 Surface wax and coatings

Harvested horticultural commodities exhibit excessive weight loss as a result of moisture loss via transpiration and to a lesser degree the loss of carbon via respiration, reducing the shelf life and fruit quality (Purvis, 1983; Mannheim and Soffer, 1996; Johnston and Banks, 1998). The application of surface waxes and coatings have been found to address this problem, encompassing both physiological and aesthetic effects. Surface coatings or waxes impart a gloss to the exterior of the fruit, thereby contributing to their aesthetic appeal (Nisperos-Carriedo *et al*., 1990, Maftoonazad and Ramaswamy, 2008). More importantly, waxes are able to reduce fruit weight loss when applied to the exterior by creating a partially permeable layer. This layer reduces the rate at which moisture is able to escape from the fruit to the surrounding environment, thus maintaining a higher moisture content (Hagenmaier and Baker, 1993). The permeability of the wax layer also contributes to a reduced rate of gas exchange between the fruit and the surrounding environment, lowering the respiration rate (Hagenmaier and Shaw, 1992; Hagenmaier and Baker, 1993; Johnston and Banks, 1998; Maftoonazad and Ramaswamy, 2008). The reduced moisture loss ensures that fruit cells remain turgid, consequentially promoting fruit firmness.

Nisperos-Carriedo *et al*. (1990) found that coated oranges exhibited increased concentrations of volatile compounds (acetaldehyde, ethyl acetate, and methyl butyrate), contributing to enhanced orange juice flavour, compared to uncoated fruit. Similar findings were noted by Nisperos-Carriedo *et al*. (1991). Chitosan coatings are a form of active packaging in which deposits from the film are transferred to the fruit surface, aiding in the inhibition of fungal growth (Chien *et al*., 2007). Purvis (1983) observed that waxed orange and grapefruit displayed greater loss in moisture and a reduction in the acidity, compared to individually sealed fruit. Hagenmaier and Baker (1994) found that natural carnauba wax was more effective in reducing weight loss in citrus, compared to shellac or polyethylene waxes. At present, shellac, carnauba and polyethylene waxes are commonly used for citrus (Dodd *et al*., 2008). Hagenmaier and Shaw (1992) recommended that a suitable citrus wax have high oxygen, carbon dioxide and ethylene permeabilities, while having low water vapour permeability. This will allow for a reduced transpiration rate without excessively restricting the respiration rate. However, some of the disadvantages of wax coatings are off-flavours and odours associated with impaired

oxygen and carbon dioxide exchange. This leads to anaerobic respiration, resulting in the release of malodorous organic acids and increased ethanol and acetaldehyde concentrations (Cohen *et al*., 1990; Hagenmaier and Shaw, 1992; Hagenmaier and Baker, 1993; Mannheim and Soffer, 1996; Njombolwana *et al*., 2013). In addition, kumquat fruit are consumed with the skin. As a result, consumers may not be willing to purchase kumquat fruit with waxes or chemical residues on the surface. Table 9.3 (Appendix A) lists some of the surface coatings applied to citrus fruit.

2.5.3 Ultra-violet irradiation

Ultra-violet (UV) radiation from the sun can be divided into three groups, UV-C (below 280 nm), UV-B (280-320 nm) and UV-A (320-390 nm) as described by Stapleton (1992). Studies by Kim *et al*. (1991), Rodov *et al*. (1992), Rodov *et al*. (1994) and D'hallewin *et al*. (2000) have found that the release of two phytoalexins, (1) scoparone and (2) scopoletin, were elicited by UV light. These compounds contribute to the fruits' resistance against pathogens. Effective UV-C dosage of fruit ranges from 0.25 $kJ.m^{-2}$ to 8.0 $kJ.m^{-2}$ (Terry and Joyce, 2004; Palou *et al*., 2008). Stevens *et al*. (1996) reduced the onset of green mould in grapefruit and tangerines, and stem-end rot and sour rot in tangerines, by hormetic exposure of the fruit to 0.84 $kJ.m^{-2}$ to 3.6 $kJ.m^{-2}$ of UV-C. Similarly, D'hallewin *et al*. (2000) found that grapefruit exposed to 0.5 $kJ.m^{-2}$ of UV-C irradiation developed less decay than untreated control fruit. Stevens *et al*. (1996) found the effectiveness of UV-C irradiation in reducing postharvest decay was due to its germicidal effect on the fruit surface and its ability to induce fruit resistance (Stevens *et al*., 1996). However, Rodov *et al*. (1994) attributed the fruit decay inhibition of UV irradiation to induced fruit resistance rather than to any germicidal effect because the sample citrus fruit were inoculated with the pathogens after exposure to UV light. In addition to a pathological defence, UV-irradiated fruit were shinier and firmer, possibly due to tissue lignification (Ben-Yehoshua *et al*., 1992). However, excessive amounts of UV irradiation can result in damage in kumquat that appears as peel damage and excessive shrivelling of the peel as observed by Rodov *et al*. (1992; 1994). Similar observations were made by Ben-Yehoshua *et al*. (1992) on lemons. Canale *et al*. (2011) found that UV irradiation was able to inhibit CBS. Table 9.4 in Appendix A lists some of the effects of UV-C irradiation on different citrus cultivars.

2.5.4 Chlorinated water

Hypochlorite has been used widely as a disinfectant for controlling postharvest pathogens in fruit and vegetables (Delaquis *et al*., 1999; Prusky *et al*., 2001; Workneh *et al*., 2003). Hypochlorite in chlorinated water is available as chlorine gas, calcium hypochlorite, or sodium hypochlorite (Boyette *et al*., 1993). A hypochlorite concentration ranging from 55-70 $mg.L^{-1}$ at a temperature of 40°C and pH of 7.0 is generally recommended for treating fruit and vegetables (Boyette *et al*., 1993). Kitinoja and Kader (1994) recommend a pH of 6.5 to 7.5. Chlorination is a dynamic process and requires constant monitoring of factors, such as pH, hypochlorite concentration, temperature, organic matter, time, and the growth stage of the pathogen as explained by Boyette *et al*. (1993).

Mango dipped in 100 $\mu g.mL^{-1}$ chlorinated water for 600 seconds (10 minutes) resulted in a higher marketability after storage, which could be attributed to the disinfectant property of hypochlorite (Tefera *et al*., 2007). Delaquis *et al.* (1999) found that warm chlorinated water (47°C for 180 seconds) was more effective in retarding both the development of spoilage microorganisms and the onset of the brown discolouration in iceberg lettuce, compared to cold water. A 10-second wash using 200-250 $mg.L^{-1}$ free chlorine of lettuce reduced the *Listeria monocytogenes* population by a factor of 10 (Simons and Sanguansri, 1997). However, chlorine can possess phytotoxic properties (bleaching or burning) due to high concentrations of either calcium or sodium with sodium hypochlorite being slightly more phytotoxic than $Ca(OCl)_2$ (Suslow, 1997; Jowkar, 2006). Workneh *et al*. (2003) observed slight bleaching of carrots dipped in chlorinated water (100 $\mu g.mL^{-1}$). In addition, the disadvantage of chlorine is the instability of the chlorinated compounds, resulting in a loss and change in concentration (Premuzic *et al*., 2007). Korf *et al*. (2001) found that chlorine dioxide (10 $\mu g.mL^{-1}$) was more effective in reducing conidial germination in citrus fruit, compared to calcium hypochlorite (100 $\mu g.mL^{-1}$). Gil *et al*. (2009) stated that a washing time exceeding 60 or 120 seconds had no significant effect in reducing the bacterial count. However, Boyette *et al*. (1993) found that long dips were more effective than quick dips. A spray of water containing 800-1000 $mg.L^{-1}$ hypochlorite was used to disinfect Nagpur mandarins and Mosambi sweet oranges with the aid of nylon brushes (6-8 seconds) (Ladaniya, 2008). Smilanick and Sorenson (2001) used chlorinated water (50 $mg.L^{-1}$) at 1350 kPa for 45 seconds and a delivery rate of 2400 $L.min^{-1}$ for washing of lemons. Research regarding the effect of chlorinated water on kumquat fruit

is limited. Currently, the South African kumquat industry uses a 1% chlorine bath or chlorine dioxide (ClO_2) as a pre-treatment (Beghin, 2014c). Therefore, there exists the potential for optimising disinfection treatments for kumquat fruit in South Africa. Some of the hypochlorite treatments applied to citrus fruit are appended as Table 9.5.

2.5.5 Anolyte water

Electrochemically activated water (ECA) or anolyte water is produced by the electrolysis of a salt and water solution (Bakhir, 1997; Leonov, 1997; cited by Workneh *et al*., 2003; Buck *et al*., 2002; Whangchai *et al*., 2010; Workneh and Osthoff, 2010; Workneh, 2014). During this process the molecular state of water is changed from stable to metastable where two types of ECA water are produced, (1) anolyte and (2) catholyte water. The anolyte water, which has an oxidation-reduction potential (ORP) of +1000 mV, is better suited for disinfecting due to its antimicrobial characteristics and the catholyte, which has an ORP of -800 mV, is preferred for its cleaning and detergent ability. The active compound of anolyte water is the hypochlorous acid. A comparison of the effect of anolyte water and chlorinated water on carrots revealed that the latter resulted in a greater loss of firmness and physiological weight (Workneh *et al*., 2003). However, both chlorinated and anolyte water were effective in reducing the microbial flora of carrots. Carrots dipped in the anolyte water also appeared to be shinier and smoother. Similar findings were obtained by Workneh *et al*. (2011) when treating tomatoes in which lower counts of yeast and mould were detected. However, chlorinated water resulted in a lower coliform count than anolyte water. Guentzel *et al*. (2010) found that a dip and daily spray of electrolyzed oxidizing water at a pH of 6.3-6.5 at 250 $mg.L^{-1}$ and an ORP of 800-900 mV reduced the onset of gray mould and brown rot in grapes and peaches, respectively. Unpublished studies by Lesar (2002) found that Neutral Anolyte also known as ACTSOL (Radical Waters, Johannesburg, South Africa) was comparable to chlorine (200 $mg.L^{-1}$) in preventing green mould and sour rot spore germination. Dilutions of Neutral Anolyte at 1:5 and 1:10 and exposure times of 30, 60, 300 and 600 seconds appeared to be effective. The immersion of tangerines for 480 seconds in electrolyzed oxidizing water was the most effective in reducing infection caused by *P. digitatum* (Whangchai *et al*., 2010). Buck *et al*. (2002) recommend the use of anolyte water for disinfection due to it being environmentally safe and effective. Research regarding the effect of anolyte water on kumquat fruit is limited.

2.5.6 Sodium carbonate and sodium bicarbonate

The application of sodium carbonate (SC) or sodium bicarbonate (SB) solutions to the exterior of citrus fruit acts as a disinfectant specifically to reduce the postharvest incidence of green mould (Smilanick *et al*., 1997). The efficacy of SC and SB can be attributed to their high pH levels suppressing the action of these pathogens (Venditti *et al*., 2005), as well as promoting the host defence response (Youssef *et al*., 2014). Smilanick *et al*. (1997) found that oranges immersed in 4% or 6% (w/v) SC solutions heated to 40.6°C or 43.3°C for 120 seconds resulted in the most effective control of green mould. Clementine mandarins dipped for 150 seconds in a 3% SC solution at 50°C displayed a significant inhibition in blue and green moulds (Palou *et al*., 2002). Mandarins dipped in 2% or 3% SC solutions at room temperature for 60 seconds or 150 seconds resulted in a 40-60% reduction in both blue and green mould. The disadvantage of SB is that heating of these solutions results in the release of carbon dioxide and a subsequent decrease in the pH (Smilanick *et al*., 1999). In addition, Obagwu and Korsten (2003) found that SB treatment (5%) of oranges resulted in salt burn on the peel. Table 9.6 (Appendix A) lists some of the SC and BC treatments applied to citrus fruit.

2.5.7 Postharvest biocontrol treatments

Microbial biocontrol (microbial antagonists) has been used successfully to control the postharvest decay of many horticultural commodities as an alternative to chemical based synthetic treatments (Huanga *et al*., 1995; El-Ghaouth *et al*., 2000; Ippolito *et al.*, 2000; Droby *et al*., 2009). Wisniewski and Wilson (1992) and Sharma *et al*. (2009) described the two methods of using micro-organisms to control postharvest decay as to either (1) use and control the already existing favorable microflora on the fruit surface or (2) to introduce foreign antagonists to postharvest pathogens. The biocontrol mode of action of yeasts are based on competing for nutrients and space, inducing fruit resistance and the production of lytic enzymes (Arras, 1996; Ippolito *et al.*, 2000; Bar-Shimon *et al*., 2004), while bacterial antagonists rely on the production of antibiotics (Wisniewski and Wilson, 1992). The combined use of biocontrol agents with other treatments has been found more beneficial to the fruit, compared to biocontrol as the only treatment, as seen in Table 9.7 contained in Appendix A. Some of the biocontrol products that are commercially

available include BioSave-110®, Boniprotect® and BioSave-111® (Workneh *et al*., 2003; Bar-Shimon *et al*., 2004; Abraham *et al*., 2010; Lahlali *et al*., 2011). A study by Abraham *et al*. (2010) revealed the preventative action of yeast strains B13 and Grape in controlling green mould decay in oranges and lemons in South Africa. Similar positive results were obtained by Arras (1996). However, Droby *et al*. (1998) found that biocontrol was not as effective as the only mode of postharvest treatment in alleviating decay in citrus on a commercial scale. The limitation of applying biocontrol agents commercially is primarily the 'uncontrolled' postharvest environment, compared to laboratory applications (Wisniewski and Wilson, 1992). Research is required to determine the suitability of biocontrol agents, such as yeast B13, for commercialization (Abraham *et al*., 2010). Furthermore, there is no research specifically on the effects of biocontrol agents on kumquat, which warrants research being undertaken in this area.

2.5.8 Integrated pre-packaging treatments

The application of combined treatments, as opposed to individual treatments, have been found to be far more effective in maintaining citrus fruit quality and preventing decay (Obagwu and Korsten, 2003; Sen *et al*., 2007). Hot water treatment, hypochlorite and salt treatments do not offer a permanent solution to postharvest decay but rather their effects have a limited duration (Hong *et al*, 2007). Therefore, other treatments need to be applied to provide prolonged fruit protection. The combination of hot water and chlorine was shown to be effective in reducing the onset of decay in citrus fruit. The addition of a biocontrol further improves the efficacy (Korf *et al*., 2001; Sen *et al*., 2007). Similarly, the treatment of chlorine and hot water proved to be beneficial in mandarins (Sen *et al*., 2007). Ben-Yehoshua *et al*. (2005) found the treatment of oranges with hot water dipping (52°C for 120 seconds) followed by UV irradiation resulted in reduced fruit decay. Table 9.8 in Appendix A presents some of the effects of integrated pre-packaging treatments applied to citrus fruit. It is evident from the table that biocontrol agents are more effective when used in combination with other pre-packaging treatments in reducing fruit decay.

Based on the reviewed literature it can be suggested that an effective combination of treatments makes use of (1) disinfection; (2) curative and (3) preventative modes of action (Laing, 2014c). Chlorine (hypochlorite) or anolyte water provides a disinfecting effect. Curative treatments include hot water, surface coatings or waxes, or SC or SB (Laing,

2014c). Preventative treatment methods include biocontrol agents, such as B13. Disinfection treatments have the ability to remove existing pathogenic microorganisms present on the fruit surface. Curative treatments are able to 'repair' and initiate fruit resistance. The preventative mode of action hinders potential infection. Few studies have dealt with the combined action of a disinfectant, plus a curative and preventative treatment on citrus fruit.

2.6 Harvest and Postharvest Technologies and Machinery

Studman (2001) refers to postharvest technology as the handling, sorting, storage, transportation, managing and marketing of horticultural products from the point of harvest until consumption. This section discusses technologies and machinery typically used during the postharvest handling of citrus fruit, with a focus on pre-packaging treatments.

2.6.1 General packhouse operations

The purpose of a packhouse is for the effective and efficient application of postharvest treatments and the facilitation of transport and distribution of fruit to the required markets (Tugwell, 1988). Figure 9.1 in Appendix A illustrates a general packhouse processing line for citrus. The components of each processing line may vary, depending on the citrus cultivar and product end-use. A U-shaped layout incorporating all the handling processes is considered to be an efficient design in a packhouse, preventing cross-contamination (Kitinoja and Kader, 1994).

2.6.1.1 Transport to the packhouse

After harvest citrus fruit are transported by road to packhouses commonly by trucks or tractors and trailers (Ladaniya, 2008; Beghin, 2014a). Due to the small volume of kumquat fruit harvested in South Africa, compared to other citrus varieties, groupage or mixed loading is used. This method allows for different fruit to be transported in a single vehicle. TransFresh Corporation (1999) has developed a mixer guide allowing users to input specific produce to determine their transport compatibility. According to the mixer guide, kumquat is suitable to be transported with oranges, mandarin and avocados but not

with banana, mangoes or tomatoes. Currently kumquats are transported in non-refrigerated vehicles to packhouses that are located a distance away from the orchards (Beghin, 2014a). This time lapse and non-refrigerated transport result in increased fruit temperatures, leading to fruit deterioration (Brosnan and Sun, 2001). Sullivan *et al*. (1996) stated that every minute after harvest is vital with regard to the quality of fresh fruit. Hence, the removal of field heat immediately after harvest is desirable (Dennis, 1984; Brosnan and Sun, 2001). Other sources of damage during transport include compression damage due to overloading, non-fruit inclusions such as debris, as well as rough roads.

2.6.1.2 Types of conveyors

Conveyors assist in moving produce through process lines and are important elements in any packhouse. Kitinoja and Kader (1994) describe three types of conveyors used in the fresh fruit industry: (1) belt conveyor, (2) push-bar conveyor and (3) roller conveyors. The belt conveyor merely moves the produce in one direction without rotation, the push-bar conveyor rotates the fruit forward while moving in the forward direction, and the roller conveyor rotates the fruit backwards while moving in the forward direction. These conveyors can be modified depending on the produce and process stage. Kitinoja and Kader (1994) suggest using foam-padded ramps and shallow slopes for the transition from different conveyors. If only steep slopes are possible, then drapes or curtains should be used over the sloping sections, and slow conveyor speeds should be adopted (exact timing depends on the process). Pourdarbani *et al*. (2013) used a belt conveyor of 300 mm long and 45 mm wide, driven by an inverter-driven half a horsepower electro-gearbox (15 Hz) to sort date fruit based on their maturity stage. The speed achieved was 22.6 $m.min^{-1}$. However, the belt conveyor only exposed one side of the fruit, which was not an accurate basis to determine the stage of maturity. Pourdarbani *et al*. (2013) recommended using a conveyor that exposed all sides of the fruit. Garcia-Ramos *et al*. (2003) used a chain conveyor composed of rollers with two truncated cones to hold individual fruit moving at a speed of 1 $m.s^{-1}$ powered by a variable speed electric motor. This ensured that an impact sensor could make direct contact with each fruit to determine the firmness.

2.6.1.3 Citrus sorters and graders

Initial inspection and sorting is essential to ensure that fruit passing through processing lines and to the customer is of an acceptable quality. This process also allows fruit to be grouped according to their specific end-use. Manual sorting, inspection and classification are subjective and may vary depending on personnel and even the time of day (Aleixos *et al*., 2002; Ladaniya, 2008). Personnel involved in sorting and grading must be adequately trained to classify fruit based on colour, size, blemishes, and shape. Commercial manual sorters are composed of aluminium rollers of varying widths (1 200-1 500 mm) and capacities (2-6 tons.h^{-1}), depending on the fruit being conveyed. The rollers rotate on their axis to expose the entire surface of the fruit for better inspection. The sorting area should be adequately illuminated with white light. Sorting and pre-sizing are often performed simultaneously for greater efficiency. Mechanical sorters include the drum roller, which has a series of holes of a specific diameter. The fruit are rotated inside the drum, causing fruit of a smaller diameter to exit the drum through the smaller holes. This method merely sorts citrus fruit based on size, and not on defects or internal fruit quality (Kim *et al*., 2004). This system is best suited for round fruit. A more advanced method combines visible and non-visible (near infra-red, ultra-violet and fluorescent) spectra of light to classify fruit and to detect defects (Blasco *et al*., 2009).

2.6.1.4 Combined washing and disinfection treatments

Washing is required to remove field dirt, superficial mould, field heat and any chemicals or fungicides from the fruit peel (Petracek *et al*., 1998). Washing may use potable water (rinsing) or the addition of disinfection chemicals (Gil *et al*., 2009). Washing systems include closed flumes, such as pipes, open flumes such as channels, baths and wash tanks (Simons and Sanguansri, 1997), or conveyors and nozzles (Fallik *et al*., 1999). Hypochlorite is the most common disinfectant used in the horticultural industry. Simons and Sanguansri (1997) and Laing (2014c) recommend a chlorine solution pH within the range of 6.5-7.2. Rinsing after disinfection allows for excess disinfecting agents to be removed from the fruit surface. Smilanick and Sorenson (2001) rinsed lemons with potable water at 10 mL of water per fruit after treatment with a liquid lime sulphur solution. Batch washing can be used to clean fruit as they move along the length of the bath. However, only one side of the fruit is exposed to the water because the fruit floats with one side up. In addition, the temperature of the water may vary in different zones of the tank if a powerful water circulation system is not installed (Fallik, 2004; Laing,

2014b). An efficient system will, therefore, treat the entire area of the fruit under the recommended treatment conditions, such as temperature, concentration and time. The water in batch washing may become laden with foreign material from the fruit and lose its effectiveness to wash, which contaminates the fruit (Simons and Sanguansri, 1997).

Washer units with brushes and nozzles remove field dirt as well as some of the natural fruit wax. Soft bristle brushes are suited for fruit with a delicate skin, such as limes, lemons and mandarins (Ladaniya, 2008). It is essential that the brushes be saturated with water to reduce damage to fruit, compared to a dry brush method. Ladaniya (2008) recommended horsehair roller brushes at a speed of 100 rpm with a brushing time of 10-20 seconds to reduce bruising. Njombolwana *et al*. (2013) found that cleaning with horsehair brushes resulted in 59% green mould sporulation, compared to 64% with synthetic polyethylene brushes. Petracek *et al*. (1998) found that washing grapefruit, oranges and tangelos using a roller brush and high water pressure nozzles (1380-2760 kPa) for 10 seconds removed the epicuticular wax. However, no detrimental effects on the mass loss or moisture and gas exchange were identified. Systems implementing nozzles and brushes require a shorter operational time, compared to immersion systems (Fallik, 2004). This allows for more fruit to be processed.

2.6.1.5 Combined washing and hot water treatments

Fallik *et al*. (1999) combined hot water treatment with rinsing to treat sweet peppers. The fruit move along a set of brushes, while simultaneously passing under hot water applied through nozzles, thus cleaning and disinfecting the fruit. Rinsed and heat-treated sweet peppers were firmer, cleaner and displayed less decay when exported, compared to dry brush cleaning. Fallik *et al*. (1999) also found that this method sealed cracks in the fruit epidermis, promoting a longer shelf life. Hot water rinsing and brushing offered a shorter exposure time of 10-30 seconds, compared to dipping or immersion (Irtwange, 2006). This equipment has also proven to be beneficial for citrus fruit (Porat *et al*., 2000). A minimum exposure time of 20 seconds at 56°C inhibited the germination of green mould. Fallik (2004) recommended that additional research be undertaken to explore the effects of hot water brushing technologies on horticultural commodities to reduce the reliance on pesticides. Additional benefits of hot water treatments are presented in Section 2.5.1.

2.6.1.6 Biocontrol and fungicide application

The application of microbial antagonists can be successfully achieved by postharvest dips or sprays (Sharma *et al*., 2009). The incorporation of biocontrol agents and fungicides into waxes and coatings has also been used in commercial packing lines (Wisniewski and Wilson, 1992; Ladniya, 2008; Sharma *et al*., 2009; Fan *et al*., 2014). Ladaniya (2008) suggested that combining fungicides with waxes reduces the antifungal action of the fungicide. Furthermore, the residue is often greater when the fungicide is included within the wax. However, the benefit of combining the wax and fungicide is to avoid having two separate operations. Brown *et al*. (1983) found that dipping treatments were more effective than spraying due to the ability of aqueous solutions to penetrate cracks in fruit, where pathogens are most prevalent. However, Ladaniya (2008) found that these dipping methods promoted disease and contamination. The preferred application method uses nozzles, which distributes the solution in a fine mist as the fruit pass on a conveyor belt. Altieri *et al*. (2013) devised a method of applying imazalil fungicide via an imazalil thin film treatment unit. The equipment is made of a stainless steel slide (1270 × 700 mm) that allows for the free flow of the fruit. A 30 litre tank supplies the imazalil solution via a centrifugal pump. An overflow blade controls the film thickness. A separating surface then allows excess solution to be drained and finally the fruit is dried using a centrifugal fan.

2.6.1.7 Surface waxing and coating methods

The choice of type, consistency, viscosity and other characteristics of waxes vary depending on the fruit and the objective of applying the wax (Hall, 1981). The manner in which the wax is applied to the fruit also varies. Some waxes are applied using wool felt while the fruit are rotated on roller brushes. The wool felt extends across the width of the belt and a polyethylene sheet prevents evaporation of the wax from the felt (Kitinoja and Kader, 1994). Waxes can also be applied using a single traversing hydraulic nozzle (Ladaniya, 2008). The nozzle moves every 1-1.5 seconds. The horsehair roller brushes carrying the fruit are saturated with the wax while the metered nozzle releases a fine spray of wax over the fruit. The wax application is metered using a pump, and is atomised with compressed air to create the fine spray. Other wax applicators make use of a manifold

incorporating a number of fixed nozzles mounted above roller brushes (Hall, 1981). This method does not require mechanical movement.

2.6.1.8 Surface moisture removal

The removal of moisture from the fruit surface is one of the main unit operations in citrus processing (Fito *et al*., 2004). The air temperature has a profound effect on the fruit quality. Excessively high temperatures can result in dry patches and extreme moisture loss. Grierson and Smith (1986) and Ladaniya (2008) recommended that temperatures should not exceed 54°C. Air can either pass over heaters and be directed on to the fruit as they roll along conveyors, or air can be drawn through heaters located below the fruit. Centrifugal fans can also be used (Altieri *et al*., 2013). Tugwell (1988) recommended high velocity cool air flows to dry fruit because it is more efficient, compared to hot air. Drying commonly follows waxing in commercial citrus packhouses. Fito *et al*. (2004) observed that as more wax was applied to citrus fruit, higher air velocities or air temperatures resulted in a shorter drying time. Oranges coated with 0.024 $kg.m^{-2}$ of wax required a 20 second drying time at 1 $m.s^{-1}$ (25°C), compared to a drying time of 10 seconds at 2 $m.s^{-1}$ (25°C). Grierson and Smith (1986) recommended mechanical methods to remove excess water, compared to using heated air, to conserve energy. Mechanical methods include sponge rubber rollers or horsehair brushes with a rotation of no more than 75 rpm or 100 rpm for 10-20 seconds (Grierson and Smith, 1986; Ladaniya, 2008). Cool air is preferred because heated air, together with the rolling action of the brush, may damage the fruit. Currently in South Africa kumquats are air dried following a chlorine treatment, using ambient air as they move along a conveyor belt (Beghin, 2014c). The current handling of kumquat fruit is further discussed in Section 2.7.3.

2.6.2 Energy sources and consumption during operations

The current energy crisis in South Africa has placed great pressure on the fruit industry, particularly with export fruit. International markets demand that suppliers demonstrate environmentally sustainable practices. Electricity has been identified as the main source of energy to power the various postharvest processes at the packhouse (Bouwer, 2011). Within a packhouse, processes using conveyors, water pumps, dryers, sorting tables,

carton machinery, and lights are the most energy intensive operations (Bouwer, 2011). A benchmarking analysis undertaken in 2010 by Bouwer (2011) revealed that energy consumption varied among South African packhouses from 15 $kW.h.ton^{-1}$ to 44 $kW.h.ton^{-1}$. Bouwer (2011) recommended that more energy efficient equipment as well as management practices be applied to conserve energy.

Miller and Singh (1986) identified the principle categories of energy to be electricity, boiler fuel (fuel oil or natural gas) and refined oils (gasoline or liquid petroleum (LP)). Electricity is primarily used for lighting and packing line machinery, transport vehicles use gasoline or LP and boilers mainly require oil or natural gas. The average energy utilisation in Florida citrus packhouses using (1) electricity, (2) fuel oil and/or natural gas and (3) gasoline and/or LP equated to 321.3 $kJ.kg^{-1}$, 313.3 $kJ.kg^{-1}$ and 40.1 $kJ.kg^{-1}$, respectively. The California study by Miller and Singh (1986) revealed that low grade heat was generally used due to air and water temperatures being limited to 70°C and 40°C, respectively. Studies by Ozkan *et al.* (2004) and Waheed *et al*. (2008) have found diesel to be one of the main fuel sources in the citrus supply chain. The use of diesel ranged from tractor operations on the field to the generation of electricity in the packhouse. The logistics involved in the fruit industry mainly consumes energy in the form of diesel for vehicles, and bunker fuel oil and marine diesel for shipment (Browne *et al*., 2008).

Recycling of materials, such as water during postharvest handling saves energy (Boyette *et al*., 1993). However, precautions need to be taken to prevent further decay in using contaminated water, by using filters or screens. Reducing the pressure on packhouses to process large volumes of fruit could reduce the energy requirements. Therefore, alternative methods of citrus processing could assist in this regard. A shift from non-renewable to renewable forms of electricity may also provide a viable research opportunity for implementation in the future.

In addition to diesel and electricity, water is a major input in the processing of citrus fruit (Thevendiraraj *et al*., 2003). The amount of water varies, depending on the end product. A water mass balance for a citrus juice plant conducted by Thevendiraraj *et al*. (2003) revealed a total fresh water consumption of 240.3 $t.h^{-1}$ and a waste water generation of 246.1 $ton.h^{-1}$. Water intensive operations include rinsing, washing, disinfection and hot water treatment. Recycling the water from these operations, by installing filters, could

reduce the large amount of water used, creating a more efficient system. Balls (1986) found that the water requirement for (1) a rotary barrel (deluge), (2) conveyor, (3) conveyor and pre-soak and (4) rotary barrel (immersion) ranged from 2.7-5.5 $m^3.t^{-1}$, 3.5-5.5 $m^3.t^{-1}$, 1.0-2.0 $m^3.t^{-1}$ and 0.2-0.4 $m^3.t^{-1}$, respectively.

2.7 The South African Citrus Supply Chain and Markets

This section focuses on the South African citrus market, specifically that of export, since South Africa is a major exporter of citrus fruit. Included in this section are the key factors required to improve the South African supply chain, with a focus on kumquat fruit.

2.7.1 The main citrus cultivars in South Africa

The citrus industry is the third largest horticultural industry in South Africa, contributing 15% of the total gross value of horticultural crops of R53.2 billion during the 2013/14 season, compared to R6.9 billion during the 2010/11 season (Department of Agriculture, Forestry and Fisheries, 2012; 2015a). The four predominant categories of citrus in South Africa are oranges, soft citrus, grapefruit and lemons (van Dyke and Maspero, 2004; Department of Agriculture, Forestry and Fisheries, 2012; 2015a). According to the Department of Agriculture, Forestry and Fisheries (2015a) in 2014 oranges accounted for the largest portion of the citrus cultivars planted of 70%. This was followed by grapefruit with 16%, lemons and limes of 12% and soft citrus at 2%. There is limited cultivation of kumquats in South Africa, with only 30 hectares dedicated to growing kumquats in the northern and eastern regions.

2.7.2 Market access

The South African citrus industry is primarily export-driven (Dodd *et al*., 2008; Department of Agriculture, Forestry and Fisheries, 2012; 2015a; Ntombela and Moobi, 2013). The European Union (EU) is the main recipient of the South African citrus exports, with smaller export markets such as Russia, Thailand, South Korea, China, Indonesia and Japan (Department of Agriculture, Forestry and Fisheries, 2012; Citrus Growers' Association, 2013; Department of Agriculture, Forestry and Fisheries, 2015a). Approximately 4-5 tons of kumquats are harvested per annum, with Europe (including

the United Kingdom), Mauritius and United Arab Emirates being the main export destinations (Begin, 2014c). The demand for kumquats has been fairly stable over the past five years. India has been targeted as a potential market; however, high tariff charges pose a challenge. The EU-Commission Implementing Regulation (2011) contains the marketing standards for citrus fruit including, minimum quality requirements, maturity requirements, classifications, and sizing requirements among other requirements. The level of supply of citrus to the EU is possibly unsustainable due to the demands of retailers, concerning pesticide residues (Citrus Growers' Association, 2013). By introducing new and uncommon citrus varieties, it is possible to broaden access to other international markets. The commercial citrus industry for export is well established; however, the niche for small-scale citrus farmers regarding the export market is not yet defined. The National Agricultural Marketing Council has been focussing on small-scale farmers and rural development through the Strategic Integrated Project 11 initiative (Citrus Growers' Association, 2013).

2.7.3 Supply chain challenges

Gaining access to new markets is required to increase the revenue generated by exports, and to enhance international partnerships. However, the strict standards imposed by export markets have proved to be a challenge because this increases the quality standards that South African citrus fruit have to achieve. Due to the limited export of citrus fruit in 2013 to the EU due to black spot, there is pressure on South Africa to make up for the lost income as well as to regain their reputation for a high standard and quality of fruit. Ortmann *et al*. (2006) found that by modelling the fruit export infrastructure, the Levubu packhouse in South Africa for soft and hard citrus represented a bottleneck in terms of the volume of fruit that needed to be processed. It was recommended that more efficient management and utilisation of existing infrastructure be implemented (Ortmann *et al*., 2006). This creates a research opportunity for other methods to be developed, such as on-farm units capable of treating citrus fruit in-field, as opposed to a conventional packhouse.

The following is an explanation of each stage in the kumquat supply chain:

1a - Harvesting: kumquats are harvested manually, as explained in Section 2.3.2. The daily yield harvested varies depending on the number of pickers and picking conditions.

Once harvested, the kumquats are then loaded onto vehicles and transported to packhouses.

1b - Transport is performed by non-refrigerated vehicles to packhouses. At the packhouse the kumquats undergo two pre-packaging treatments.

1c - Pre-packaging: the first treatment requires the fruit to be rinsed to remove the field heat and dirt. The kumquats are then disinfected with chlorine (1% chlorine solution (hypochlorite) or chlorine dioxide) and then air dried.

1d - Packaging: once treated, the kumquats are then packaged into 2 kg cardboard cartons. The kumquats are closely packed in each carton. The cartons are then stacked onto 24-ton trucks and secured in place with straps.

2 - Transport: due to the lower yield of kumquats, compared to other fruit, groupage transport is required, whereby kumquats are transported along with other horticultural commodities. Transport to the airport is carried out at night when the ambient temperatures are lower, to compensate for the absence of refrigeration. The distances from Letsitele and Levubu to the OR Tambo International Airport, in Johannesburg, are approximately 450 km and 850 km, respectively.

3 – Airport (dependant on market destination): once the kumquats arrive at the freight agents at the airport, they are then stored temporarily in a cool store room.

4 - Transport: the kumquats are then exported to international markets either by airfreight (overnight) or by sea (14 days).

5 - Export Market: the EU is the main export market; however, other markets, such as India, are being targeted.

Each stage is associated with challenges that have been identified in Table 2.1.

Table 2.1 Challenges and probable solutions in the South African kumquat supply chain

Stage	Challenges	Probable Solutions
1a	Both flowers and fruit appear on the tree at the same time.	Pickers need to be careful not to damage buds/ flowers, which can negatively affect subsequent fruit development and quality.
	The stem is not fully removed, resulting in a sharp protrusion, which can result in bruising of adjacent fruit.	Fruit can be collected in flat boxes in single layers rather than in bags (Laing, 2014a).
1b	Non-refrigerated transport, results in increased temperatures, which can be detrimental to the postharvest fruit quality (Workneh and Osthoff, 2010).	Implement refrigeration units in trucks.
		Pre-treat fruit on-site to withstand higher temperatures and reduce pathogenic infections during transit.

Stage	Challenges	Probable Solutions
1c	Fruit undergo a basic wash and chlorine disinfection. Chlorine treatments are more effective when used in combination with other pre-packaging treatments, such as hot water treatments (Boyette *et al*., 1993).	Include other suitable treatments as part of the pre-packaging process of kumquats.
2	Groupage requires kumquats to be transported with other horticultural commodities due to the small volumes harvested.	Make use of smaller transport vehicles.
		Transport kumquats with commodities that are not detrimental to the fruit.
4	Transport by sea can take up to 21 days. Extended shipping times can result in pathogenic infections, decay and quality deterioration.	Effective pre-packaging treatments need to be applied early in the supply chain to enhance the shelf life and maintain fruit quality, such as hot water treatments (Rodov *et al*., 1995), and waxes (Hagenmaier and Baker, 1994).
		Effective packaging treatments need to be applied to enhance the shelf life and maintain fruit quality.
5	There are few small-scale farmers contributing to the export market (Department of Agriculture, Forestry and Fisheries, 2012; 2015a).	Develop resources to assist small-scale farmers to contribute to export markets, such as a mobile packhouse to reduce investments in large packhouses.

2.8 Discussion and Conclusion

Citrus fruit are susceptible to microbial infections and postharvest decay once harvested. *P. digitatum* and *P. italicum* have been identified as the major pathogens affecting citrus fruit (Obagwu and Korsten, 2003; Talibi *et al*., 2012; Mokomele, 2013; Zhang, 2014). Exposure to excessive field temperatures after harvest and during transport to the packhouse promotes the onset of decay, negatively affecting fruit quality (Dennis, 1984; Sullivan *et al*., 1996; Brosnan and Sun, 2001). In addition, non-fruit inclusions and rough roads during transport may result in further damage to the fruit. Logistical delays in conveying fruit to packhouses further extends the time between harvest and processing. In South Africa the current method of transporting kumquats to packhouses employs unrefrigerated trucks, which exposes the fruit to excessive pathogenic infections and high temperatures. This increases the rate of decay as the fruit are not pre-treated prior to transport. Furthermore, mixed loading requires that the kumquat fruit be transported simultaneously with other horticultural commodities, which can be harmful to the commodities if they are not compatible (TransFresh Corporation, 1999). These factors have a direct influence on time and temperature after harvest, which affect the fruit quality and shelf life (Brosnan and Sun, 2001).

Fruit packhouses are the hub at which majority of the postharvest handling occurs, such as pre-packaging treatments, packaging and storage. This implies that fruit are typically transported some distance from the orchards to the packhouse before any treatments can be applied. Currently, the main pre-packaging treatments identified within the citrus industry are postharvest fungicides, hypochlorite disinfection and waxing (Ladaniya, 2008). However, the relative efficacy of other treatments, such as hot water, biocontrol agents and anolyte water on citrus fruit have not been fully explored. Hot water treatments have a significantly positive effect on the postharvest citrus quality, particularly kumquat fruit, in terms of reduced decay as a result of the *Penicillium* pathogens, reduced weight loss and firmer fruit, (Schirra *et al*., 1995; Ben-Yehoshua *et al*., 2000; Porat *et al*., 2000; Rodov *et al*., 2000; Fallik, 2004; Sapitnitskaya *et al*., 2006; Strano *et al*., 2014). Schirra *et al*. (1995), Ben-Yehoshua *et al*. (2000) and Rodov *et al*. (2000) found 53°C for 120 or 30 seconds to be the optimum temperature and time combination for kumquat heat treatments. Hot water treatments do not contain any chemicals and are, therefore, recommended for kumquat fruit due to the manner in which the fruit is consumed (Rodov *et al*., 1995; Schirra *et al*., 1995; Ben-Yehoshua, 2000; Schirra *et al*., 2011). Waxes were found to reduce the moisture loss and create shiny fruit surfaces; however, excessive waxing can result in the development of off-flavours due to suppressed gas exchange (Njombolwana *et al*., 2013). The use of hypochlorite as a disinfectant is common practice in the postharvest fruit industry. The current hypochlorite treatment of kumquats at packhouses in South Africa uses a 1% chlorine solution or chlorine dioxide. Biocontrol agents have been presented as an environmentally friendly alternative to fungicides. The yeast strain B13 provided positive results in preventing *P. digitatum* decay in oranges and lemons in South Africa (Abraham *et al*., 2010). Further studies are required to determine the feasibility of using B13 as a biocontrol agent for kumquat fruit. Excessive UV-C irradiation (>0.5 $kJ.m^{-2}$) or too high salt content (5%) can result in damage to the citrus fruit peel (D'hallewin *et al*., 2000; Obagwu and Korsten, 2003; Canale *et al*., 2011).

Combined pre-packaging treatments have been recommended, compared to individual treatments, due to their higher overall efficacy in reducing decay and maintaining fruit quality (Obagwu and Korsten, 2003; Sen *et al*., 2007). An effective pre-packaging treatment combination should include a disinfectant (hypochlorite or anolyte water), curative (hot water) and a preventative agent (biocontrol). Many studies have focused on combined pre-packaging treatments on citrus fruit, such as oranges and mandarins (Korf

et al., 2001; Sen *et al*., 2007). However, these treatments did not combine disinfection, curative and preventative modes of action (Ben-Yehoshua *et al*., 2005; Sen *et al*., 2007). Insufficient data is available on anolyte water as a pre-packaging treatment in citrus, particularly in kumquats. Therefore, a portion of this study was dedicated to determining the efficacy of anolyte water as a disinfectant of kumquat fruit.

The equipment used in industry focussed primarily on brushes and spray nozzles to remove dirt and clean the fruit surface. Hydraulic sprayers or water baths have been used for the application of hot water and chlorine treatments. Fallik (1999) developed a hot water brushing and rinsing unit for sweet peppers. However, this equipment was aimed at operation within a packhouse. Electricity and diesel were found to be the main sources of energy in packhouses. The energy utilisation among South African packhouses varies from 15 $kW.h.ton^{-1}$ to 44 $kW.h.ton^{-1}$ (Bouwer, 2011). Efficient equipment and management practices are essential to introduce and manage energy saving.

Ideally, an in-field integrated pre-packaging unit would address these concerns by pre-treating the fruit on-site, immediately after harvest, a concept that has not been previously documented. The unit could incorporate disinfection, curative and preventative pre-packaging treatments. This unit could be described as 'condensing' the packhouse processes into a mobile unit that could be operated on-site at the orchard. Mobile units for small-scale farmers are likely to reduce their financial investment in large packhouses. South African kumquat yields are lower than other major citrus varieties, such as oranges (Beghin, 2014c). This allows for the unit to be taken directly to the orchard, where treatment of small quantities of fruit can be carefully managed. More importantly, the damaging delay between harvest and pre-packaging treatments would be greatly reduced, which would improve fruit quality and reduce decay.

Innovative and convenient techniques of treating kumquat fruit after harvest are required to reduce losses that occur when the fruit is transported untreated to packhouses. It is envisioned that by developing the South African kumquat industry, a larger export market can be created, as well as providing small-scale kumquat farmers with a niche in this export arena. The availability of literature pertaining to the pre-packaging treatment of kumquat fruit, particularly in South Africa, is limited. Therefore, postharvest kumquat

research is required to improve and extend the shelf life, by developing an in-field pre-packaging treatment unit.

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3. PHYSICAL PROPERTIES OF KUMQUAT, ORANGES, GRAPEFRUIT AND LEMONS REQUIRED FOR POSTHARVEST EQUIPMENT DESIGN

3.1 Abstract

The physical properties of Nagami kumquat, Navel oranges, Star Ruby grapefruit and Eureka lemons were investigated to provide design parameters required for the efficient design of the integrated postharvest citrus treatment unit (IPCTU). The physical properties that were investigated included the fruit mass, length, width, thickness, volume, geometric diameter, surface area, sphericity, shape index and fruit density. The mass of kumquats, oranges, grapefruit and lemons were found to be 15.68 g, 340.84 g, 374.13 g and 130.00 g, respectively. The mean surface area was determined to be 2 727.62 mm^2, 25 197.88 mm^2, 28 176.37 mm^2 and 13 499.35 mm^2 for kumquats, oranges, grapefruit and lemons, respectively. The mean volume occupied per fruit was found to be 15.00 mL, 388.80 mL, 474.44 mL and 127.6 mL for kumquats, oranges, grapefruit and lemons, respectively. The shape of the fruit varied based on the shape index, from oval for kumquats and lemons to round for oranges and oblate for grapefruit. The fruit mass, dimensions, surface area, shape (sphericity and shape index) and fruit density were considered as the most pertinent parameters required for the design of agricultural machine and equipment. This data is recommended for use in designing postharvest processing machinery.

3.2 Introduction

The South African citrus industry is largely export based, with the European Union and United Kingdom being the primary export destinations (Department of Agriculture, Forestry and Fisheries, 2014). From 2007 to 2011, approximately 64% of citrus produced in South Africa was exported (Ntombela and Moobi, 2013). Kumquat fruit do not contribute to the main citrus cultivars produced in South Africa. However, there is growth in the popularity of kumquat fruit, with around 30 hectare producing 180 000 cartons of this exotic fruit (Beghin, 2014). The Nagami kumquat cultivar is the most popular cultivar consumed and exported from South Africa. Orange fruit are the most produced citrus fruit

in South Africa, making it economically and industrially important (Sharifi *et al*., 2007; Department of Agriculture, Forestry and Fisheries, 2012; 2015; Citrus Growers' Association, 2013; 2014). This is followed by grapefruit, lemons and limes and soft citrus. In 2013 exports of Navel oranges, Star Ruby grapefruit and Eureka lemons amounted to 2.5 million; 200 000 and 70 000 tons, respectively (Citrus Growers' Association, 2014). South Africa is considered as the largest exporter of oranges in the world and is ranked third in the global production of grapefruit, following China and USA, respectively (Ntombela and Moobi, 2013).

The physical properties of fruit are the most essential aspect required for grading, transfer, and processing systems (Topuz *et al*., 2005; Yehia *et al*., 2010; Jaliliantabar *et al*., 2013). Mohsenin (1986); cited by Sharifi *et al*. (2007), identified the fruit mass, fruit dimensions (length, width, and thickness), volume and surface area to be of great importance in the design of equipment. Akubuo and Odigboh (1999) determined the physical properties of egusi fruit in order to efficiently design coring equipment. Yehia *et al*. (1999) designed mandarin grading equipment based on the physical and mechanical properties of the fruit. The physical and mechanical properties of cantaloupe were also determined for the design of seed extraction equipment (Yehia *et al*., 2010). The rate of processing as well as the load imposed on the equipment by the fruit can be established based on the physical properties. The physical properties provide an indication of the movement and behaviour of the fruit during processing (Sharifi *et al*., 2007). The physical properties of Nagami kumquat fruit in Iran were obtained by Jaliliantabar *et al*. (2013). Topuz *et al*. (2005) determined the physical and nutritional properties of Alanya, Finike, W. Navel and Shamouti oranges in Turkey. Sharifi *et al*. (2007) investigated the physical properties of Tompson oranges in Iran. The physical properties of Seedless Lisbon and Frost Eureka lemons were obtained by Baradaran *et al*. (2014) and sweet lemons by Taheri-Garavand and Nassiri (2010) in Iran. Research involving the physical properties of other fruit such as dates, tomato and gumbo has also been conducted (Akar and Aydin, 2005; Jahromi *et al*., 2008; Taheri-Garavand *et al*., 2011).

Although there has been extensive research conducted on citrus fruit, literature pertaining to the physical properties of citrus fruit in South Africa are limited, with a particular deficiency on kumquat fruit. Therefore, this study aimed to determine the physical properties of kumqut fruit, orange, grapefruit and lemons in South Africa so as to assist

in the efficient design of agricultural machine and equipment. Consequentially, this could promote a more effective citrus supply chain and improved fruit quality.

The objective of this study was to determine the physical properties of Nagami kumquat, Navel oranges, Star Ruby grapefruit and Eureka lemons in South Africa, to aid in the design process of the postharvest citrus treatment unit in Chapter 6.

3.3 Materials and Methods

3.3.1 Sample fruit

Nagami kumquat (*Fortunella margarita*), Navel oranges (*Citrus cinensis*), Star Ruby grapefruit (*Citrus paradisi*) and Eureka lemons (*Citrus limon*) were selected as the sample fruit as these are the most common citrus fruit produced in South Africa. Fifty fruit of each cultivar were sampled (Topuz *et al*., 2005; Sharifi *et al*., 2007; Jaliliantabar *et al*., 2013). These were obtained from a commercial market in Pietermaritzburg, KwaZulu-Natal in September 2014. The fruit were then transported to the University of KwaZulu-Natal Food Science and Agricultural Engineering Laboratory, located approximately 10 km away.

3.3.2 Data collection and analysis

The physical properties that were determined included the fruit mass, the external dimensions (length, width, thickness) and the fruit volume. This was conducted under laboratory conditions at an ambient temperature of 23°C. From these parameters, the geometric diameter, surface area, sphericity, shape index and fruit density were calculated. The mean and standard deviation of the data were obtained using Microsoft Excel 2010 spreadsheet software (Jaliliantabar *et al*., 2013). The following sections describe the methods applied in determining the measured and calculated fruit physical properties.

3.3.2.1 Measured physical properties

The mass of each fruit was determined using an electronic Avery Berkel scale (Avery Berkel, England, United Kingdom) at an accuracy of 0.1 g. The volume of the fruit was determined by the displacement of water method using a 1 000 mL graduated beaker (Sharifi *et al*., 2007; Jaliliantabar *et al*., 2013). The length (L), width (W) and thickness (T) were the three major external linear dimensions that were measured with the aid of a digital Mitutoyo Vernier calliper (Mitutoyo Corporation, Kanawa, Japan) (Topuz *et al*., 2005; Jahromi *et al*., 2008; Fawole *et al*., 2013; Jaliliantabar *et al*., 2013). The dimensions L, W and T are normal to each other as indicated in Figure 3.1.

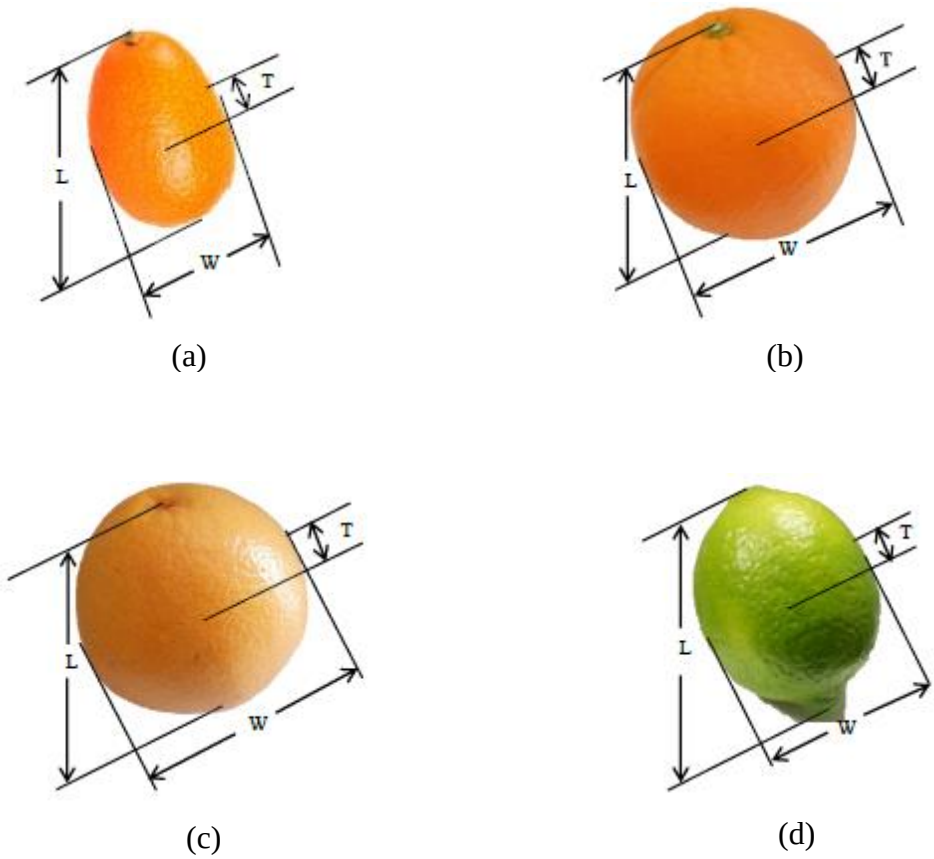

Figure 3.1 The length (L), width (W) and thickness (T) of kumquat fruit (a), orange fruit (b), grapefruit (c) and lemon (d)

3.3.2.2 Calculated physical properties

Based on the measured parameters, the geometric diameter (D_g, mm), surface area (S_F, mm^2), sphericity (Ø), shape index (SI) and the fruit density (ρ_f, $kg.m^{-3}$) were calculated using equations (3.1), (3.2), (3.3), (3.4) and (3.5), respectively (Ku *et al*., 1999; Sharifi *et al*., 2007; Jaliliantabar *et al*., 2013).

$$D_g = (L \times W \times T)^{\frac{1}{3}} \quad (3.1)$$

where

D_g, L, W and T are as previously stated.

$$S_F = \pi \times Dg^2 \quad (3.2)$$

where

π = PI, 3.14159

$$Ø = Dg / L \quad (3.3)$$

$$SI = L/(W \times T)^{1/2} \quad (3.4)$$

$$\rho_f = (M/1000)/V \quad (3.5)$$

where

M = fruit mass [g]

V = volume of fruit [m^3]

3.4 Results and Discussion

3.4.1 Fruit mass and dimensions

The measured and calculated physical properties of the sample fruit are presented in Table 3.1 together with the standard deviation and coefficient of variation. The mean mass for the kumquat fruit was determined to be 15.68 g, similar to the mass of Nagami kumquat from Iran of 14.30 g (Jaliliantabar *et al*., 2013). The dimensions (L, W and T) were also comparable to those obtained by Jaliliantabar *et al*. (2013). The mean mass for the orange

fruit was found to be 340.84 g. The mass determined by Sharifi *et al*. (2007) for the largest grade of Tompson oranges was 268.28 g and that found by Topuz *et al*. (2005) was 271.40 g for Navel oranges. The mean length, width and thickness of the orange samples were 91.15 mm, 88.23 mm and 89.39 mm, respectively. These dimensions are larger than those reported by both Sharifi *et al*. (2007) and Topuz *et al*. (2005). The mass of grapefruit was found to be 374.13 g, with a length, width and thickness of 88.87 mm, 98.31 mm and 97.29 mm, respectively. Similarly, Sinclair (1972) measured the average mass of Marsh grapefruit from California to be within a range of 259 g to 375 g and a diameter between 80 mm to 100 mm. The mean mass of the lemons has been found to be 130.00 g, compared to 122.28 g for Frost Eureka lemons and 105.12 g for sweet lemons (Taheri-Garavand and Nassiri, 2010; Baradaran *et al*., 2014). The mass is an important parameter as it determines the amount of weight imposed on the unit specific processing capacity, which consequentially affects the dimensions of the unit.

3.4.2 Fruit volume and density

Jaliliantabar *et al*. (2013) documented the volume of the kumquat to be 12.30 mL, compared to 15 mL for the current study. The volume for the grapefruit was 474.44 mL, compared to 388.80 mL for oranges and 127.6 mL for lemons. The fruit density calculated for the grapefruit (790.83 $kg.m^{-3}$), which was less than that for oranges (877.76 $kg.m^{-3}$), lemons (1 028.60 $kg.m^{-3}$) and kumquats (1 116 $kg.m^{-3}$). A similar observation was made by Sharifi *et al*. (2007), where the largest size classification of Tompson oranges displayed the lowest fruit density. Due to the smaller size of the kumquat fruit, compared to the orange, grapefruit and lemons, it has a higher fruit density. A higher fruit density means that more fruit can be processed during a predetermined time period, assuming all the variables such as the conveyor speed of the equipment is constant. Similar values for the density of orange and lemon fruit were calculated by Topuz *et al*. (2005) of 903.15 $kg.m^{-3}$ and Baradaran *et al*. (2014) of 1010 $kg.m^{-3}$, respectively.

Table 3.1 Measured and calculated physical properties of Nagami kumquat fruit, Navel oranges, Star Ruby grapefruit and Eureka lemons

Parameter	Kumquat		Navel Oranges		Star Ruby Grapefruit		Lemon	
Measured Parameters	Mean (SD)	CV (%)	Mean (SD)	CV (%)	Mean (SD)	CV (%)	Mean (SD)	CV (%)
Mass (g)	15.68 (±1.83)	11.67	340.84 (±21.81)	6.40	374.13 (±29.49)	7.88	130.00 (±10.93)	8.41
Length (mm)	37.48 (±1.79)	5.37	91.15 (±4.84)	5.30	88.87 (±3.88)	4.36	78.76 (±5.90)	7.49
Width (mm)	26.42 (±1.62)	7.04	88.23 (±4.92)	5.57	98.31 (±4.19)	4.26	59.69 (±1.91)	3.20
Thickness (mm)	26.42 (±1.62)	7.04	89.39 (±2.29)	2.56	97.29 (±3.03)	3.11	59.57 (±2.28)	3.83
Volume (mL)	15.00 (±4.24)	4.24	388.80 (26.35)	6.78	474.44 (±40.78)	8.59	127.60 (±15.72)	12.32
Calculated Parameters								
Geometric Diameter (mm)	29.43 (±1.52)	5.17	89.52 (±2.73)	3.05	94.68 (±2.12)	2.24	65.39 (±2.42)	3.71
Surface Area (mm^2)	2727.62 (±281.51)	10.32	25197.88 (±1522.42)	6.04	28176.37 (±1259.29)	4.47	13449.35 (±1009.15)	7.50
Specific Gravity	1.12 (±0.32)	28.78	0.88 (±0.04)	4.70	0.79 (±0.05)	6.94	1.03 (±0.11)	10.73
Sphericity	0.79 (±0.05)	10.32	0.98 (±0.04)	4.70	1.07 (±0.04)	3.40	0.83 (±0.04)	4.77
Shape Index	1.14 (±0.13)	9.28	1.03 (±0.06)	5.78	0.91 (±0.05)	5.21	1.32 (±0.09)	7.14
Density ($kg.m^{-3}$)	1116.00 (±321.14)	28.78	877.76 (±41.22)	4.70	790.83 (±54.85)	6.94	1028.60 (±110.34)	10.73

SD, Standard deviation; CV, Coefficient of variation

3.4.3 Fruit shape

The shape index for the kumquat, oranges, grapefruit and lemons samples were 1.14, 1.03, 0.91 and 1.32, respectively. According to Bahnasawy *et al*. (2004), a shape index > 1.5 is an indication of spherical fruit, while values < 1.5 are indicative of oval fruit. Jaliliantabar *et al*. (2013) classified kumquat fruit as oval due to the shape index being less than 1.5. However, according to Combrink *et al*. (2013) a shape index of 1, > 1 or < 1 is an indication of a perfectly round, oval or oblate (slightly flattened at the poles) fruit, respectively. The description of the shape index as used by Combrink *et al*., (2013) appears to be more wide-ranging, compared to that of Bahnasawy *et al*. (2004). Therefore, kumquat and lemon fruit can be described as having an oval shape, oranges as being round to slightly oblate and grapefruit as being oblate.

The shape of the fruit will affect the fruit movement and behaviour in equipment. It is easier for round objects to roll along the conveyor. However, oval or oblate fruit can move irregularly. This is of particular importance in equipment that applies different treatments to fruit surfaces such as waxes, since a uniform application will be required. To accommodate this, roller conveyors can be used to promote the uniform movement of fruit (Pourdarbani *et al*., 2013). Combrink *et al*. (2013) described orange fruit as round to slightly oblate, grapefruit as oblate and kumquat and lemon as oval. Whereas according to Bahnasawy *et al*. (2004), all the sampled citrus cultivars would be spherical in shape, which is not an accurate description. The sphericity of kumquat, oranges, grapefruit and lemons were 0.79, 0.98, 1.07 and 0.83, respectively. Comparable values for oranges and lemons were found by Topuz *et al*. (2005) of 0.98 and by Baradaran *et al*. (2014) for lemons of 0.85. Kumquats were found to have a sphericity of 0.75 (Jaliliantabar *et al*., 2013).

Thermal treatments such as hot water dipping and blanching are extensively used in postharvest fruit processing (Wang *et al*., 2001). The fruit shape and size have a significant influence on the rate of heat transfer within the fruit, affecting the exposure time (Wang *et al*., 2001). Conventional heating methods rely on the convective heat transfer from the heating medium to the fruit surface and then by the conductive heat transfer from the surface to the centre of the fruit (Wang *et al*., 2001). Conductive heat

transfer requires a longer time due to the lower thermal diffusivity of fruit (1.6×10^{-7} $m^2.s^{-1}$), compared to metals ($1.5\text{-}17 \times 10^{-5}$ $m^2.s^{-1}$). As a result, larger fruit may require an even longer time for the centre of the fruit to reach the desired temperature. However, for the purpose of this research, the temperature at the core of the fruit was not a concern.

3.4.4 Surface area

The mean surface areas for kumquat, orange, grapefruit and lemon fruit were found to be 2 727.62 mm^2, 25 197.88 mm^2, 28 176.37 mm^2 and 13 499.35 mm^2. The surface area is a significant factor, particularly in postharvest equipment, which applies different treatments to the fruit surfaces. These can include water for rinsing, hot water, disinfecting materials or waxes. The amount of substances required for processing can be determined by surface area of the fruit. This implies that a larger surface area will require more treatment materials.

3.5 Conclusion

The mean mass for the kumquat, orange, grapefruit and lemon samples were found to be 15.68 g, 340.84 g, 374.13 g and 130.00 g, respectively. This is an important parameter as it determines the force imposed on the equipment. Fruit density was calculated to be 1 116.00 $kg.m^{-3}$, 877.76 $kg.m^{-3}$, 790.83 $kg.m^{-3}$ and 1028.60 $kg.m^{-3}$ for kumquat, orange, grapefruit and lemon fruit, respectively. The largest sampled cultivar, being grapefruit, was observed to have the lowest fruit density. Fruit density can have an influence on the quantity of fruit processed. Fruit with higher densities as in the case of kumquats and lemons, compared to oranges and grapefruit can be assumed to be processed in larger quantities for a given process. Orange fruit was found to be round to only slightly oblate, grapefruit was found to be oblate and lemons were found to be oval. Fruit shape can have an effect on the manner in which the fruit move within processing equipment. The fruit shape also influences the rate of heat transfer during thermal treatment, where larger fruit will require a longer time to reach the desired temperature. The results obtained from this study were used in the design process of an IPCTU, further described in Chapter 6.

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4. *IN VITRO* AND *IN VIVO* DISINFECTION AND BIOCONTROL TREATMENTS TO REDUCE DECAY CAUSED BY *PENICILLIUM DIGITATUM* AND *PENICILLIUM ITALICUM* IN KUMQUAT FRUIT

4.1 Abstract

Current trends in food regulations recommend more environmentally friendly treatments to fully, or partly, replace synthetic chemicals and fungicides used to maintain the postharvest quality of fruit. This study focused on the use of anolyte water (150 $mg.kg^{-1}$), chlorinated water (150 $mg.kg^{-1}$), a *Candida fermentati* yeast isolate (B13) and B13 in combination with anolyte water or chlorinated water to reduce the incidence of *Penicillium digitatum* and *P. italicum* in kumquat fruit *in vitro* and *in vivo*. The treatments were found to be highly significant ($P \leq 0.001$) in inhibiting the growth of *P. digitatum*. Chlorinated water combined with B13 resulted in the greatest zone of inhibition of *P. digitatum* under *in vitro* conditions of 11 mm. *In vivo* studies revealed that anolyte water only and anolyte water combined with B13 resulted in shinier and smoother fruit with less moisture loss, compared to other treatments. After 14 days of storage under ambient conditions, a section of the fruit was transferred to rose Bengal agar. Anolyte water combined with B13 was the most effective treatment in reducing the proliferation of *P. digitatum*, whereas B13 only and chlorinated water combined with B13 were effective in reducing the proliferation of *P. italicum*. Neither anolyte water nor chlorinated water had a detrimental effect on B13. Combinations of B13 with a disinfectant solution provided the best control of fungal decay and can be recommended as pre-packaging treatments for kumquats.

4.2 Introduction

Postharvest decay in citrus fruit is an important factor affecting the quality and marketable value of citrus products. Droby *et al*. (1998), Ladaniya (2008), Gomez-Sanchis *et al*. (2012), Altieri *et al*. (2013), and Youssef *et al*. (2014) have identified *Penicillium digitatum* and *P. italicum* as the most severe postharvest fungal pathogens affecting citrus fruit. *P. digitatum* and *P. italicum* can be described as wound parasites, which require a

wound as shallow as 0.25 mm for infection (Erasmus *et al*., 2015; Fallanaj *et al*., 2016). These pathogens are ubiquitous and capable of infecting citrus fruit at each stage of the supply chain (orchard, packhouse, storage and market distribution) via the dissemination of spores in the air. Research has largely focused on *P. digitatum*. However, *P. italicum* is of similar importance due to its ability to grow at lower temperatures, compared to *P. digitatum* (Brown, 1994; Erasmus *et al*., 2015). This is of particular importance during storage and shipping in which protocols require fruit to be at temperature of less than 10°C and in the case of kumquat fruit a temperature of 4.5°C is required during transport (Askarne *et al*., 2012; Department of Agriculture, Forestry and Fisheries, 2015; Erasmus *et al*., 2015). Fungicides have commonly been used to control these postharvest pathogens. However, more environmentally friendly treatment alternatives are required due to the development of fungal resistance to fungicides and the growing public demand for safer foods (Ben-Yehoshua *et al*., 2005; Zhang and Swingle, 2005; Zhang, 2007). Some of these treatments include hot water, biocontrol agents and anolyte water (Ben-Yehoshua *et al*., 2005; Workneh *et al*., 2011). Anolyte water has been found to possess strong germicidal effects on most pathogens (Al-Haq *et al*., 2002). During the generation of anolyte water, a salt (sodium chloride) and water solution is passed through a special unit, which converts the molecules from a stable state to a metastable state via the process of electrolysis (Workneh *et al*., 2003; Whangchai *et al*., 2010). The anode and cathode are separated by a non-selective membrane. Anolyte water is produced at the anode and is characterised by an oxidation-reduction potential in the range of +1000 mV and the presence of hypochlorous acid.

Additional research is required to establish pre-packaging treatments that rely less on synthetic chemicals and fungicides, and more on natural treatments. However, the author was not able to find published literature that focuses on the effect of integrated treatments on the microbiological quality of kumquat fruit. Therefore, this study was aimed at determining the effect of different treatments (chlorinated water, anolyte water and a biocontrol agent) to control *P. digitatum* and *P. italicum* in kumquat fruit, using both *in vitro* and *in vivo* assays. The *in vivo* study was necessary to compare the findings to that of the *in vitro* study. It would also assist in ascertaining the effect on the physical appearance of the fruit when subjected to different treatments.

The specific objectives formulated for this study were to determine:

1. The most effective disinfectant treatment (chlorinated water or anolyte water) to inhibit the growth of *P. digitatum* and *P. italicum*.
2. The most effective treatment or combination of treatments to reduce decay due to *P. digitatum* and *P. italicum* in kumquat fruit.
3. The disinfecting effect of chlorinated water or anoyte water on the biocontrol agent.

4.3 Materials and Methods

4.3.1 Sample fruit production

Nagami (*Fortunella margarita*) was identified as the sample fruit, being the main kumquat variety exported from South Africa. Kumquat fruit samples were obtained from the Letsitele region, just outside Tzaneen, and Levubu near the Kruger National Park, Limpopo Province, South Africa. The kumquat orchards are registered with the Department of Agriculture, Forestry and Fisheries and are clear of citrus black spot and fruit fly. Rooister Boerdery and Premier Fruit Exports (Pty) Ltd provided the necessary samples for testing. After harvest commercially mature kumquat fruit were couriered overnight to the UKZN laboratories. This was to ensure minimal fruit exposure to temperature fluctuations between harvesting and sampling. A total of 3 kg were used for this experiment.

4.3.2 Fungal cultures

The fungal cultures used in this study were *P. digitatum* and *P. italicum* isolated from citrus. Pure cultures of *P. digitatum* and *P. italicum* were prepared by and purchased from the Agricultural Research Council - Plant Protection Research Institute, Pretoria, South Africa and delivered in sealed potato dextrose agar (PDA) Petri dishes.

4.3.3 Preparation of inoculum

All laboratory utensils and apparatus were sterilized for 15 minutes at 121°C using a vertical type steam sterilizer (Model HL-340) (Laboratory Supplies, Durban, South

Africa). Procedures were carried out aseptically next to a flame within a laminar flow unit. Each of the Petri dishes containing the fungal culture were flooded with 20 mL of sterile distilled water (Smilanick *et al*., 1999). The conidia were then loosened with the aid of a laboratory glass rod. The conidial suspensions were passed through muslin and collected in a sterilized glass jar with Tween 20 (Uni Laboratory, South Africa) added as a surfactant (0.05 mL per 50 mL). Conidia suspension concentrations were quantified using a Neubauer Improved Haemocytometer (Hirschmann, Eberstadt, Germany) and then diluted to the desired concentration of 1×10^4 conidia.mL^{-1} using sterilized distilled water (Abraham *et al*., 2010).

4.3.4 Treatment preparation

The treatments for this study included two disinfection treatments of anolyte water and chlorinated water and a yeast biocontrol agent, which was a strain of *Candida fermentati* (B13). The treatments were as follows:

1. Anolyte water at a concentration of 100 mg.kg^{-1} (A).
2. Chlorinated water (calcium hypochlorite) at a concentration of 100 mg.kg^{-1} (B).
3. *C. fermentati* yeast isolate - B13 biocontrol agent (C).
4. Anolyte water and B13 (D).
5. Chlorinated water and B13 (E).
6. Tap water (F).

Commercially available anolyte water was obtained from Radical Waters (Johannesburg, South Africa) delivered in plastic containers to avoid loss of the ionized properties of the solution. The 100 mg.kg^{-1} chlorinated water was prepared by adding 22.06 g of calcium hypochlorite granules (Frexus CH, Arch Chemicals, Bloemfontein, South Africa) per 100 litres of tap water. This quantity was adjusted to 10 litres for this experiment. The presence of freely available chlorine in the tap water is discounted as negligible at < 5 mg.kg^{-1}. The freely available chlorine concentrations and pH of the treatment solutions was measured using Hydrion chlorine test strips and a Hydrion pH and sanitizer test kit (MicroEssential Laboratory, Inc., Brooklyn, USA), respectively.

Yeast B13, a strain of *C. fermentati* was supplied by Plant Health Products (Pty) Ltd (Nottingham Road, South Africa), on a grain substrate and packaged in a porous fabric.

The recommended concentration of B13 is 100 g per 100 litres of warm water (25-27°C), which was adjusted to accommodate 10 litres of water for this experiment.

4.3.5 Sample preparation

Untreated kumquat fruit were inspected based on uniformity of size, colour and damage (Hong *et al*., 2007). Fruit that showed signs of damage or deformity were discarded. The fruit were then thoroughly rinsed in a plastic strainer under running tap water to remove any dirt, debris or soil prior to treatments. After rinsing the fruit were dried using laboratory paper towels. The fruit were sorted in to 6 batches of 18 fruit and labelled at the base of the fruit using a white marker. Of these batches, 3 batches were inoculated with *P. digitatum* and the remaining 3 batches were inoculated with *P. italicum*.

4.3.6 *In vitro* experiment

Rose Bengal (with chloramphenicol) agar (Oxoid, Basingstoke, England) was used to culture the fungi. The agar was prepared by adding 16 g per 500 mL distilled water and autoclaved (121°C/ 15 minutes). Three replications per treatment were performed for each fungal inoculum. Diffusion disks of 0.65 mm diameter were prepared from Whatman® filter paper and autoclaved. 0.1 mL of each of the prepared inoculum was transferred aseptically onto the agar and evenly spread. Forceps were aseptically used to transfer the diffusion disks into the required treatment solution/s before being evenly positioned onto the plates to form a triangular shape. Three disks per plate were used. The plates were incubated at 25°C for five days. The diameters for the zones of growth inhibition around the disks were measured in mm (Espina *et al*., 2011).

4.3.7 *In vivo* experiment

4.3.7.1 Inoculation of kumquat fruit

Three replications, each comprising of three fruit per treatment, were performed for each fungal culture. A portion of each of the sample kumquat surfaces, near the pedicel, were disinfected with 70% ethanol. This area was selected for uniformity and for easy detection of the wounded site for inoculation. A laboratory scalpel was used to create a wound 2

mm deep. Care was taken to avoid piercing the fruit albedo (Abraham *et al*., 2010). The wounds were allowed to dry before the fruit were divided into two batches. Each fruit of the first batch was inoculated with 10 uL of *P. digitatum* inoculant at a concentration of 1×10^4 conidia.mL^{-1}. The second batch of kumquat fruit were inoculated with *P. italicum* at the same concentration. The fruit were stored at ambient conditions for two weeks to observe mould growth each day.

4.3.7.2 Isolation of fungi from infected fruits

After a period of 14 days the microorganisms on the surface of the fruits were isolated on rose Bengal agar following the method used by Sivakumar *et al*. (2012). The plates were then incubated at 28°C and mould formation was observed after 3 days.

4.4 Statistical Analysis and Data Collection

Statistical analysis was performed using the GenStat software, 17th Edition. The differences between treatments were determined by an analysis of variance (ANOVA) and the means were separated using the Duncan's Multiple Range Test, with a significance level of 0.05 (Droby *et al*., 1998; Workneh *et al*., 2011). The diameter (mm) of the zone of inhibition per plate was measured in two directions perpendicular to each other using a ruler (Espina *et al*., 2011).

4.5 Results and Discussion

4.5.1 *In vitro* experiment

Table 4.1 presents the zones of inhibition for each of the pathogens for the current study. The treatments were found to be very significantly ($P \leq 0.001$) different in inhibiting the growth of *P. digitatum* and significantly ($P \leq 0.05$) different in inhibiting the growth of *P. italicum*. Anolyte water only and tap water did not result in any inhibition of the fungal cultures. Chlorinated water combined with B13 resulted in the highest zone of inhibition of 11 mm, compared to any other treatment of *P. digitatum*. Anolyte water combined with B13 resulted in a zone of inhibition of 9 mm. Zones of inhibition of *P. italicum* for anolyte water combined with B13, chlorinated water combined with B13 and B13 alone were not

significantly different. Although Al-Haq *et al*. (2002) demonstrated the positive influence of anolyte water in inhibiting decay by *Botryosphaeria berengeriana* in pear fruit, the author was unable to find published literature on the effect of anolyte water or chlorinated water combined with a biocontrol agent on the microbiological quality of citrus fruit. The combined effect of the disinfectant with the biocontrol agent was more effective in reducing decay caused by both fungal pathogens.

Table 4.1 The zone of inhibition (mm) for *Penicillium digitatum* and *Penicillium. italicum* as a result of different treatments under *in vitro* conditions

Treatments	**Fungal Culture**	
	Penicillium digitatum	***Penicillium italicum***
Anolyte water	0^{a}	0^{a}
Chlorinated water	2^{ab}	4^{a}
Anolyte water + B13	9^{bc}	22^{b}
Chlorinated water + B13	11^{c}	26^{b}
B13	6^{abc}	24^{b}
Tap water	0^{a}	0^{a}
Significance		
Treatments	**	*
CV (%)	12.9	32.0

*, ** Significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n = 3). CV, Coefficient of variation; +, 'combined with'.

Figures 4.1 and 4.2 illustrate the zones of inhibition of *P. digitatum* and *P. italicum*, respectively. The appearance of colonies of the yeast strain B13 (*C. fermentati*) is visible in Petri dishes C, D and E in each of the Figures 4.1 and 4.2 as these Petri dishes included B13 in the treatments. This indicated that neither the anolyte water nor the chlorinated water inhibited growth of the biocontrol agent.

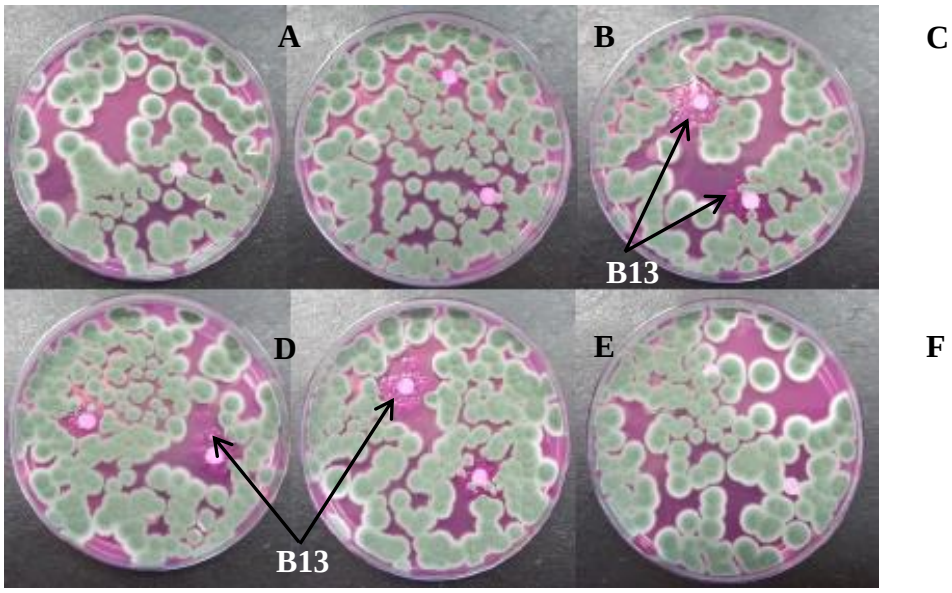


Figure 4.1 Some examples of zones of inhibition of *Penicillium digitatum* around diffusion disks treated with A, anolyte water only; B, chlorinated water only; C, B13 only; D, combined anolyte water and B13; E, combined chlorinated water and B13; F, tap water. All treatments were plated in triplicates

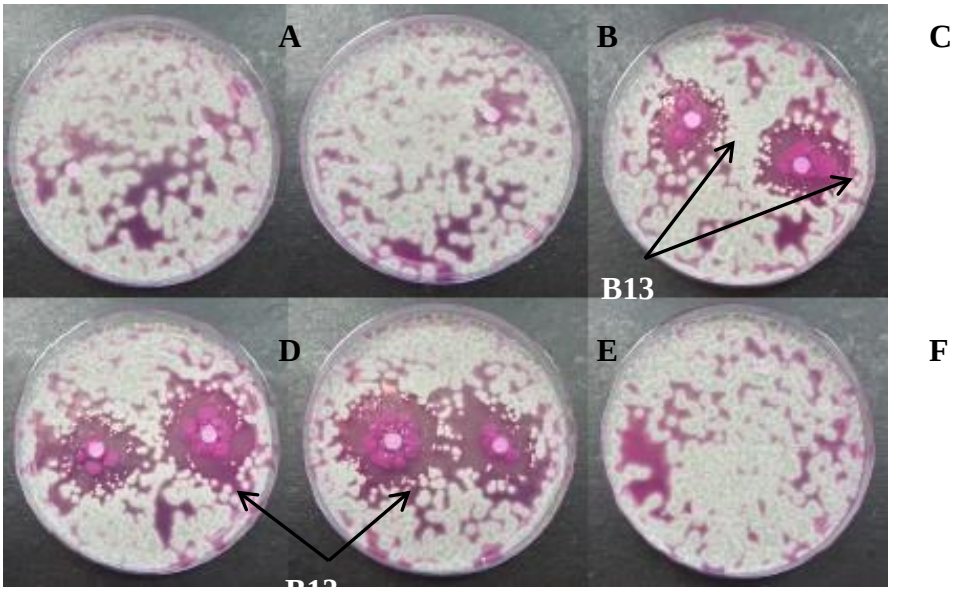


Figure 4.2 Some examples of zones of inhibition of *Penicillium italicum* around diffusion disks treated with A, anolyte water only; B, chlorinated water only; C, B13 only; D, combined anolyte water and B13; E, combined chlorinated water and B13; F, tap water. All treatments were plated in triplicates

4.5.2 *In vivo* experiment

4.5.2.1 Kumquat fruit

Figure 4.3 shows *P. digitatum*-inoculated fruit for each of the six treatments after 14 days of storage. None of the fruit demonstrated any visual mould formation throughout the 14-day storage period. However, fruit treated with chlorinated water only, B13 only and tap water displayed extensive shrivelling of the peel. The wrinkly appearance of the peel can be attributed to loss of moisture due to transpiration (Purvis, 1983; Chalutz *et al*., 1989). Workneh *et al.* (2011) also reported that the moisture loss in tomatoes dipped in chlorinated water was generally higher than anolyte water-treated samples. Fruit treated with tap water developed areas of darkening and softened tissue. Fruit treated with chlorinated water only, chlorinated water combined with B13 and B13 only experienced some form of bleaching as they appear lighter in colour compared to fruit treated with anolyte water only and anolyte water combined with B13. This could be an indication of phytotoxicity due to the chlorinated water (Suslow, 1997). Fruit treated with anolyte water only and anolyte with B13 have remained relatively smooth and shiny. In similar studies, anolyte water was used to treat carrots and tomatoes, which resulted in smooth and shiny fruit surfaces (Workneh *et al*., 2003; 2011). Anolyte water or chlorinated water is unable to fully penetrate the wounds and effectively eliminate fungal spores. The addition of the B13 biocontrol agent acts by competitively colonizing wounds at a faster rate than *P. digitatum* and *P. italicum* (Abraham *et al*., 2010). The biocontrol agent utilises the nutrients released from the wound, therefore there is not enough remianing nutrients for the *Penicillium* spores to germinate. This demosntrates the preventative mode of action of the B13 biocontrol agent.

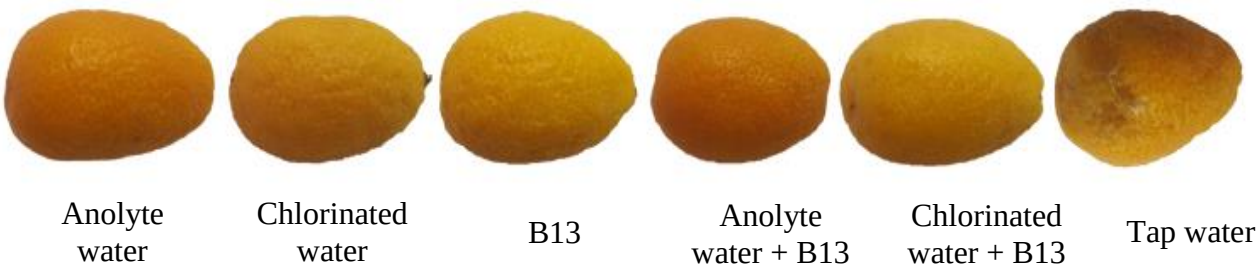


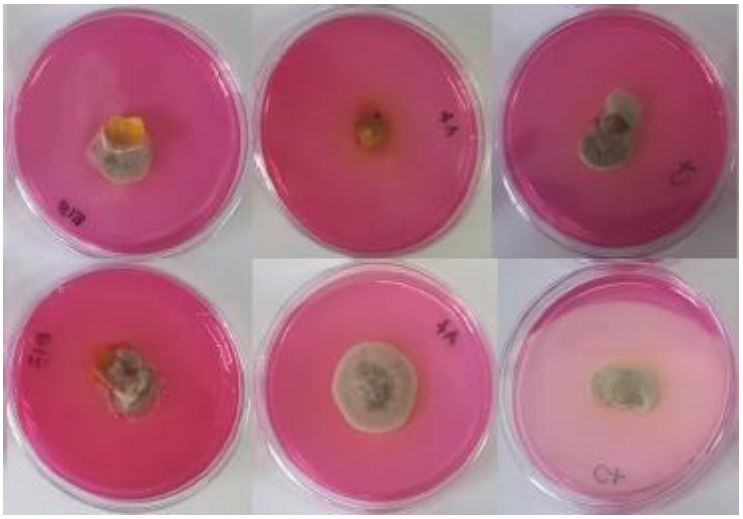
Figure 4.3 *Penicillium digitatum*-inoculuated kumquat fruit after 14 days of storage at ambient conditions

4.5.2.2 Microbial growth from plating inoculated and treated fruit

On the rose Bengal agar, anolyte water combined with B13 was the most effective in reducing the growth of *P. digitatum* as indicated in Figure 4.4. B13 alone was slightly more effective in inhibiting the growth of *P. digitatum* than chlorinated water combined with B13. However, chlorinated water combined with B13 and B13 alone were slightly more effective in reducing the growth of *P. italicum*, compared to anolyte water combined with B13. Anolyte water is an effective disinfectant as it contains free radicals with a high biocidal activity (Whangchai *et al*., 2010; Workneh *et al*., 2011). This study went further to show that the combined effect of anolyte water with B13 appeared to be more effective than the combination of chlorinated water and B13 when applied to fruit, and has potential as a pre-packaging treatment for maintaining the postharvest quality of kumquat fruit.

Penicillium digitatum

Penicillium italicum



B13 | Anolyte water + B13 | Chlorinated water + B13

Figure 4.4 *Penicillium digitatum* and *Penicillium italicum* growth on rose Bengal agar after three days of incubation at 28°C from pieces of kumquat fruit

4.6 Conclusion

The *in vitro* study revealed that chlorinated water combined with B13 was most effective in reducing the growth of *P. digitatum.* Anolyte water and chlorinated proved to be more effective when combined with B13 in reducing the growth of *P. digitatum* and *P. italicum*, than with the omission of B13. However, chlorinated water possessed a slightly higher

germicidal effect than anolyte water. The *in vivo* conditions revealed that anolyte water only and anolyte water combined with B13 resulted in more aesthetically pleasing fruit, which were shinier and smoother, compared to other treatments over the 14-day storage period. Tap water resulted in fruit that had soft and darkened peel tissue, which is indicative of the early stages of mould formation and decay. Chlorinated water only, B13 only, and chlorinated water with B13, resulted in bleaching together with excessive wrinkling and shriveling of the fruit peel. After 14 days the fruit were plated and it was observed that anolyte water combined with B13 were the most effective in reducing the proliferation of *P. digitatum*. Whereas, B13 only and chlorinated water combined with B13 were more effective in reducing the growth of *P. italicum*. The appearance of colonies of B13 on plates containing anolyte water and chlorinated water revealed that the disinfection treatments did not inhibit growth of the biocontrol agent. Instead, the combined effect of the disinfecting agents with B13 proved to be effective in reducing microbial proliferation. This study revealed that despite the disinfecting property of both anolyte water and chlorinated water, they can be used in combination with B13. Further studies are required to determine the effect of chlorinated water, anolyte water and B13 on the physical, chemical and microbiological quality of kumquat fruit to realise the potential of these treatments. The results of this study led to Chapter 5, in which anolyte water, chlorinated water, B13 and an additional environmentally friendly treatment (hot water) were used to treat kumquat fruit so as to determine the most effective treatments to be incorporated in the prototype treatment unit.

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5. THE EFFECT OF DIFFERENT PRE-PACKAGING TREATMENTS ON THE QUALITY OF KUMQUAT FRUIT

5.1 Abstract

There is an increasing need for alternative and more environmentally friendly treatments to address the issue of fruit decay and minimise losses. The aim of this experiment was to determine the effect of different pre-packaging treatments (individual and integrated) on the decay severity, physiological weight loss (PWL), peel colour, peel firmness, peel moisture content (MC) and total soluble solids (TSS) of kumquat fruit. The treatments included chlorinated water (calcium hypochlorite) and anolyte water as the disinfectants, hot water as the curative treatment and a preventative biocontrol agent, B13. Half the fruit were inoculated with *Penicillium digitatum* and the remainder with *P. italicum* prior to treatment applications. Fruits were thereafter sampled on a 7-day interval during a 28-day storage period at ambient conditions (22.7°C and 54.2% relative humidity). The storage period was found to be highly significant ($P \leq 0.001$) for all quality parameters. *P. digitatum* growth was observed to be more dominant than *P. italicum*, which can be attributed to the ambient storage conditions. Fruit subjected to anolyte water, hot water and B13 displayed no visible mould formation. Fruit treated with the calcium hypochlorite disinfection exhibited the most decay of 13.62% by Day 28. The hue angle did not substantially vary among treatments for both *P. digitatum* and *P. italicum*-infected fruit. Fruit subjected to chlorine treatments showed higher PWL than anolyte treatments. Chlorinated water only resulted in a PWL of 86.17% by Day 28 for *P. digitatum*-infected fruit. Treatments significantly influenced the peel firmness at $P \leq 0.001$. Control fruit exhibited an increase in peel firmness associated with tissue lignification. Fruit subjected to anolyte water demonstrated the least variation in peel firmness in *P. digitatum*- and *P. italicum*-inoculated samples. The least increase in the peel MC was observed in fruit treated with anolyte water, hot water and B13 (31%). An increase in the TSS resulted from all treatments. However, the increase was more apparent in fruit treated with individual treatments, specifically with chlorinated water. Overall, the integrated treatments were more effective in reducing decay and maintaining the fruit quality than individual treatments. The results obtained in this study were used in the design of a postharvest citrus treatment unit explained in detail in Chapter 6.

5.2 Introduction

Kumquat fruit, like other citrus, are classified as non-climacteric (Ladaniya, 2008). Non-climacteric fruit are fully mature/ ripe when harvested and these fruit do not exhibit drastic increases in their respiration or ethylene evolution along with changes associated with maturity or ripening. However, the postharvest shelf life of kumquat fruit is relatively short due to *Penicillium* spp. that cause high levels of decay (Schirra *et al*., 2011). These fungi have been identified as the leading cause of postharvest decay in citrus fruit (Ladaniya, 2008; Youssef *et al*., 2014). Further research is needed for the control of these fungi. Few studies have focussed primarily on kumquat fruit, compared to the more common orange, grapefruit, soft citrus and lemon varieties (Hong *et al*., 2007, Sen *et al*., 2007, Hong *et al*., 2014). During the 2013/2014 South African harvest season 47.38% and 44.09% of kumquats were exported to the European Union and United Kingdom, respectively (Department of Agriculture, Forestry and Fisheries, 2014). Given that production of the main citrus cultivars (orange, grapefruit, soft citrus and lemon) is dominated by commercial farmers in South Africa, it can be recommended that small-scale farmers focus on kumquat fruit, which has the potential to become their niche product.

Many studies have dealt with pre-packaging treatments to improve quality and increase the shelf life of citrus fruit, and in particular, to lessen infection caused by *Penicillium* spp. Such treatments include fungicides, heat treatments, surface waxing, chlorine disinfection, ultra-violet (UV) irradiation and sodium carbonate or sodium bicarbonate solutions (Johnston and Banks, 1998; Ben-Yehoshua *et al*., 2005; Li *et al*., 2008; Droby *et al*., 2009; Abraham *et al*., 2010). The trend is for commonly used synthetic fungicides to be replaced by more environmentally friendly techniques such as biological control, plant-defence promoters and physical treatments such as heat treatments (Schirra *et al*., 2011). These methods are essential for kumquat fruit due to the manner the fruit is consumed, which includes the peel. Emphasis has also been placed on using chemicals within GRAS (generally regarded as safe) compounds, particular for export fruit, which need to conform with international legislation on chemical residues (Schirra *et al*., 2011).

Based on the limited research available on kumquat fruit, hot water treatments have been found to positively influence the shelf life and quality of kumquat fruit by causing the

accumulation of scoparone in the flavedo (Ben-Yehoshua *et al*., 2005; Schirra *et al*., 2011). Similar to hot water treatments, ultra-violet irradiation is believed to initiate the synthesis of the phytoalexins scoparone and scopoletin in the fruit peel (Ben-Yehoshua *et al*., 2005). Li *et al*. (2008) found that ripening and senescence of kumquat could be delayed by the application of a chitosan and $CaCl_2$ complex package coating. Chitosan was found to have alexipharmic properties of antimicrobial and disinfection when applied to injured fruit, while the calcium ion is beneficial in terms of maturity and aging of fruit. A study by Hall (1986) investigated the use of integrated pre-packaging treatments on kumquat fruit and demonstrated the improvement in fruit quality compared to individual treatments. A dual treatment of kumquat fruit with hot water treatments followed by UV-C irradiation greatly reduced decay (Ben-Yehoshua *et al*., 2005).

The commercial use of hypochlorite is the current method of disinfecting fruit surfaces. However, hypochlorite is associated with negative effects such as bleaching and increased decay (Workneh *et al*., 2011). Alternative methods of disinfection include anolyte water or electrochemically activated water (Whangchai *et al*., 2010). However, no published research is available on the effects of anolyte water on kumquat fruit.

The aim of this experiment was to investigate the effect of different pre-packaging treatments on the postharvest quality kumquat fruit.

The specific objectives formulated for this study were to:

1. Determine the effect of individual and combined pre-packaging treatments on the physical, chemical and microbiological quality of kumquat fruit.
2. Compare the efficacy of chlorinated water and anolyte water to determine which is a more suitable disinfectant.

These findings were then used to design a pre-packaging treatment unit in the proceeding chapter.

5.3 Materials and Methods

5.3.1 Sample fruit production

Nagami (*Fortunella margarita*) was selected as the sample fruit. The same procedure was followed as per Section 4.3.1. A total of 1 100 fruit (12 kg) were used for this experiment.

5.3.2 Pre-packaging treatments

The experiment was conducted under laboratory conditions at the Food Science and Agricultural Engineering Laboratory at the University of KwaZulu-Natal. The main pre-packaging treatments identified for this experiment were: (1) chlorinated water and (2) anolyte water as the disinfectant treatments, (3) hot water as the curative treatment and (4) B13 biocontrol as the preventative treatment. These treatments were selected as they encompass disinfectant, curative and preventative modes of action, respectively. The fruit were subjected to 12 treatments as follows: (1) chlorinated water; (2) anolyte water; (3) hot water; (4) B13 biocontrol agent; (5) combined chlorinated and hot water; (6) combined chlorinated water and B13; (7) combined chlorinated, hot water and B13; (8) combined anolyte water and hot water; (9) combined anolyte water and B13; (10) combined anolyte water, hot water and B13; (11) combined hot water and B13 and (12) B13.

The following procedures were adopted for the pre-treatment of the kumquat fruit:

1. Chlorinated water: a concentration of 100 $mg.kg^{-1}$ was used at a pH of 7.0-7.2 (Beuchat and Ryu, 1997; Suslow, 1997; Laing 2014). This was achieved by dissolving 0.734 g of calcium hypochlorite granules to 5 litres of deionized water at ambient temperature (Frexus CH, Arch Chemicals, Bloemfontein, South Africa). The fruit were immersed in the chlorinated water for 30 seconds. The temperature of the water was measured to be 22°C. The freely available chlorine concentrations and pH of the treatment solution was measured using Hydrion chlorine test strips and a Hydrion pH and sanitizer test kit (MicroEssential Laboratory, Inc., Brooklyn, USA), respectively. This ensured that the correct concentration and pH was attained. Upon removal the fruit were air dried.

2. Anolyte or electrochemical activated water: commercially available anolyte water from Radical Waters (Johannesburg, South Africa) was delivered in plastic containers to avoid loss of the ionized properties of the solution. 5 litres of the anolyte water at a concentration of 100 $mg.kg^{-1}$ was used with a dipping time of 30 seconds at a pH of 6-7 at a temperature of 22°C (Lesar, 2002; Louw, 2014). The concentration and pH of the anolyte water was monitored using Hydrion chlorine test strips and a Hydrion pH and sanitizer test kit, respectively. Upon removal the fruit were air dried.
3. Hot water: approximately 2 litres of water was added to a water bath and heated to 80°C to kill most of the heat sensitive micro-organisms. The temperature was then reduced to 53°C. The kumquat fruit were then immersed in the heated water for 20 seconds (Ben-Yehoshua *et al*. 2000; Porat *et al*., 2000; Schirra *et al*., 2011; Laing 2014). Once removed the fruit were air dried.
4. B13 Biocontrol: commercially available yeast B13 (a strain of *C. fermentati*) yeast formulated by Plant Health Products (Pty) Ltd (Nottingham Road, South Africa) was used. The recommended concentration was 100 g per 100 litres of warm water (Basdew, 2014). 10 g of B13 was added to 10 litres of water comprising 8 litres of cold water to 2 litres of hot water to produce a water temperature of approximately 29°C. The fruit were immersed for 60 seconds and air dried upon removal.
5. Control: fruit were dipped in potable water for 10 seconds at 23.5°C. Upon removal the fruit were left to air dry at ambient conditions.

Once the treatments were applied, the fruit were stored in their respective batches in the Food Science and Agricultural Engineering Laboratory under ambient conditions for 28 days. Three HOBO data loggers (Onset HOBO Data Logger, Massachusetts, USA) were used to measure the ambient conditions (temperature and relative humidity) of the storage area. Once the storage period had concluded, BoxCar Pro 4.3 software was used to retrieve the temperature and relative humidity data from the data loggers for analysis.

5.3.3 Experimental design

The experiments were full factorial and performed in triplicate with three replications. This was conducted on kumquat fruit inoculated with *Penicillium digitatum* and

separately for kumquats inoculated with *P.italicum*. Fruit were inoculated prior to the application of the treatments. Two surface disinfecting treatments of chlorinated water (A) and anolyte water (B) were used. One curative treatment of hot water (C) and one preventative treatment of B13 (D) was used. These treatments were applied individually and in combination of A, B, C, D, AC, AD, ACD, BC, BD, BCD, CD with a control of tap water. After treatment application the fruit were stored under ambient conditions of 23°C and 54.% relative humidity for 28 days. Fruit were sampled on Day 0, 7, 14, 21 and 28. The number of fruit required for this study was 1080. However, to accommodate for any loss as a result of fruit that would be discarded due to damage, irregular shape or colour, a total of 1100 fruit was obtained from the orchard.

5.3.4 Isolation of *Penicillium digitatum* and *Penicillium italicum* from infected fruit

All laboratory utensils and apparatus were sterilized for 15 minutes at 121°C using a vertical type steam sterilizer (Model HL-340) (Laboratory Supplies, Durban, South Africa). 10 mL of potato dextrose agar (PDA) (Oxoid, Basingstoke, England) were added to Petri dishes and allowed to solidify for one hour. The plates were then used to culture *P. digitatum* and *P. italicum*, which were isolated from infected oranges. The Petri dishes were incubated for 3 to 5 days at 28°C to promote hyphal development. Once hyphal development was complete, a 'clean' uncontaminated portion (5 mm × 5 mm) of the mould was sub-cultured from an initial colony to new PDA Petri dishes. Seven plates were used to culture *P. digitatum* and seven plates for *P. italicum*. These plates were then incubated for a further 7 to 14 days at 28°C for fungal sporulation. *P. italicum* was observed to take a longer period to develop than *P. digitatum* and as a result a further 7 days were allocated for sporulation. Once sporulation was complete, the conidia were harvested by adding approximately 20 mL of sterile distilled water to each Petri dish (Smilanick *et al*., 1999). The conidia were then loosened with the aid of a laboratory hockey stick. The conidial suspensions were collected in two sterilized glass jars for each fungal pathogen.

5.3.5 Sample preparation

The same procedure as mentioned in Section 4.3.5 was used. The fruit were sorted in to 72 batches of 15 fruit each and labelled at the base of the fruit using a white marker. Of these batches, 36 batches were inoculated with *P. digitatum* and the remaining 36 batches were inoculated with *P. italicum* as explained in Section 5.3.6.

5.3.6 Inoculation of kumquat using *Penicillium digitatum* and *Penicillium italicum*

P. digitatum and *P. italicum* conidia that had been prepared as explained in Section 5.3.4 were used. Conidial suspension concentrations were quantified using a Neubauer Improved Haemocytometer (manufactured by Hirschmann, Germany) and then diluted to the desired concentration using sterilized water. A portion of the kumquat surface, near the pedicel, was disinfected with 70% ethanol. This area was selected for uniformity and for easy detection of the wounded site for inoculation. A needle (diameter of 1.13×10^{-3} m) was disinfected using 99.9% ethanol before being used to wound the fruit, avoiding piercing the fruit albedo (Abraham *et al*., 2010). The wounds were allowed to dry for 24 hours after which half of the fruit (36 batches of 15 fruit) were inoculated with 10 *ul* of *P. digitatum* conidial suspension at a concentration of 1×10^4 conidia.mL^{-1} (Abraham *et al*., 2012). The same procedure was followed for inoculating the remaining fruit with the *P. italicum* conidial suspension. After a further 24 hours the 12 pre-treatments were applied to the fruit which were stored at ambient conditions (22.7°C and 54.2% relative humidity).

5.4 Data Collection and Analysis

The effect of the treatments on the kumquat fruit were evaluated for changes in the physical, chemical and microbiological properties of the fruit. The physical quality parameters that were investigated included the physiological weight loss, peel firmness and peel colour. The chemical quality parameters that were investigated included the peel moisture content and total soluble solids, and the microbiological quality parameter was based on the decay severity as a result of *P. digitatum* and *P. italicum*.

5.4.1 Decay severity

Decay severity was evaluated based on the measured dimensions and calculated surface area that had fungal development and expressed as a percentage of the entire surface area. The dimensions were measured using a digital Mitutoyo Vernier calliper (Mitutoyo Corporation, Kanawa, Japan). In addition, the number of fruit that had developed fungal growth per batch was calculated and expressed as a percent on each sampling interval (Hong *et al*., 2007; Abraham *et al*., 2012; Schirra *et al*., 2011).

5.4.2 Physiological weight loss

Kumquat fruit were individually weighed using a Mettler PJ 300 scale (Mettler-Toledo, Barcelona, Spain) at the start of the experiment and at the specified sampling intervals of 7 days. The differential weight loss was calculated for each sample per interval and converted to a percentage of the original fresh weight of the fruit (wet basis) (Singh and Reddy, 2006; Hong *et al*., 2007).

5.4.3 Peel colour

The peel colour was measured using a Konica Minolta CR-400 colorimeter (Konica Minolta Inc., Osaka, Japan). The instrument was calibrated using a white calibration tile and set with a C illuminant. A mean of three readings around the equatorial region per fruit was obtained. The parameters L*, a* and b* were measured (Li *et al*., 2008). The hue angle could then be calculated as described in Equation 5.1 (Choi *et al*., 2002).

$$\text{Hue angle} = \arctan (b^*/a^*) \tag{5.1}$$

5.4.4 Peel firmness

An Instron Universal Testing Machine (Model 3345) (Advanced Laboratory Solutions, Baar, Switzerland) was used in conjunction with the Instron Bluehill 2 Version 2.25 software to determine the firmness of the kumquat peel by means of puncturing the fruit surface. Individual unpeeled kumquat fruit were placed horizontally on the curved platform (stem axis parallel to plate). A probe of 1.5 mm diameter was used to perform

two punctures per fruit sample on opposite sides of the equatorial region. The cross head speed was set at 200 $mm.min^{-1}$ to travel to a depth of 12 mm. The maximum force required to puncture the fruit was taken as the exterior fruit firmness (Churchill *et al*., 1980; Valero *et al*., 1998).

5.4.5 Peel moisture content

The sample fruit were cut in half. The pulp was removed from one half of the fruit. Approximately 2 g of the peel was placed on to a piece of aluminium foil. The weight of the foil and peel were measured using a Mettler PJ 300 scale (Mettler-Toledo, Barcelona, Spain). The samples were then placed in a hot air oven at 105°C for 24 h (Jaliliantabar *et al*., 2013). Once dried after the 24-hour period, the samples were then reweighed. The peel moisture content was calculated on a wet basis (Singh and Reddy, 2006).

5.4.6 Total soluble solids

The total soluble solids expressed as °Brix was determined by extracting juice from the pulp of each fruit and placing it on the prism of the Atago digital hand-held 'pocket' refractometer (±0.2 % accuracy) (ATAGO USA Inc., Washington, USA) (Valero *et al*., 1998, Schirra *et al*., 2011). The prism was cleaned with 99.9% ethanol and then with distilled water, using a soft cloth between samples.

5.5 Statistical Data Analysis

The statistical analysis was performed by the GenStat software, 14th Edition. The differences between treatments were determined by an analysis of variance (ANOVA) and the treatment means were separated using the Duncan's Multiple Range Test, with a significance level of 0.05 (Droby *et al*., 1998; Workneh *et al*., 2011).

5.6 Results and Discussion

5.6.1 Decay severity

Table 5.1 presents the decay severity on the surface of kumquat fruit due to *P. digitatum*. The treatment and storage period had a highly significant (P≤0.001) influence on the decay severity of kumquat fruit. No visible mould growth was observed between Days 0 and 7 for all treatments. On Day 14, a notable increase in the mould formation was measured at 4.48% of the surface area of samples treated with chlorinated water only.

Table 5.1 Changes in the decay severity (%) due to *Penicillium digitatum* encountered in kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0	**7**	**14**	**21**	**28**
Chlorinated water	0.00^{a}	0.00^{a}	4.48^{ab}	8.41^{b}	13.62^{c}
Anolyte water	0.00^{a}	0.00^{a}	0.00^{a}	1.57^{a}	4.08^{ab}
Hot water	0.00^{a}	0.00^{a}	0.00^{a}	0.79^{a}	1.31^{a}
Biocontrol (B13)	0.00^{a}	0.00^{a}	1.05^{a}	1.05^{a}	3.01^{ab}
Chlorinated water + HWT	0.00^{a}	0.00^{a}	0.00^{a}	4.71^{ab}	4.71^{ab}
Chlorinated water + B13	0.00^{a}	0.00^{a}	0.00^{a}	1.36^{a}	1.36^{a}
Chlorinated water + HWT + B13	0.00^{a}	0.00^{a}	0.00^{a}	0.52^{a}	2.62^{a}
Anolyte water + HWT	0.00^{a}	0.00^{a}	1.81^{a}	1.81^{a}	1.81^{a}
Anolyte water + B13	0.00^{a}	0.00^{a}	0.22^{a}	3.50^{ab}	4.28^{ab}
Anolyte water + HWT + B13	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
HWT + B13	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	*				
CV (%)	20.6				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

On Day 28 the mould formation had grown substantially, amounting to 13.62% (Figure 5.1).

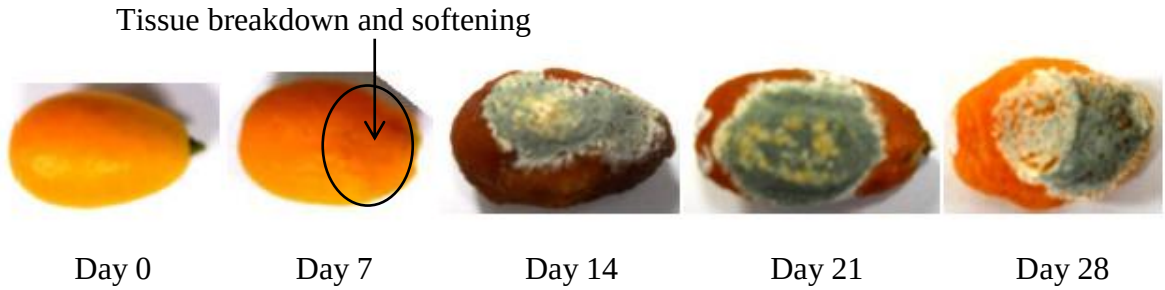

Figure 5.1 *Penicillium digitatum*-infected kumquat fruit from Day 0 to Day 28 treated with chlorinated water only

Chlorinated water alone resulted in the greatest decay severity, in which a total of 66% of the fruit in this batch displayed visible signs of mould development, as indicated in Table 5.2. This was followed by the biocontrol treatment alone with 44% of fruit displaying visible decay. This corresponds with the findings by Abraham *et al*. (2012) in which the use of the yeast biocontrol is better suited as a preventative treatment rather than as a curative treatment. The combination treatment of anolyte water + hot water + B13 did not develop any mould throughout the 28-day storage period. A similar trend was observed in the combination treatment of biocontrol with hot water as well as in the control fruit. Obagwu and Korsten (2003) also found a significant reduction in the blue and green mould of oranges due to the combination of hot water (45°C for 120 seconds) and biocontrol (*Bacillus* F1). The anolyte water and hot water treatment resulted in a mould formation of 1.81% on Day 14, which remained constant for the remaining storage period. Similarly, chlorinated water and hot water had a constant decay severity of 4.71%. Hot water only and the combination of chlorinated water and B13 resulted in low decay severity of 1.31% and 1.36%, respectively, by Day 28.

Table 5.2 Percentage of decayed fruit due to *Penicillium digitatum*

Treatment	Storage Period (Days)					*Total % of decayed fruit
	0	**7**	**14**	**21**	**28**	
Chlorinated water	0	0	22	11	33	66
Anolyte water	0	0	0	11	22	33
Hot water	0	0	0	11	11	22
Biocontrol (B13)	0	0	22	11	11	44
Chlorinated water + HWT	0	0	0	11	11	22
Chlorinated water + B13	0	0	0	11	11	22
Chlorinated water + HWT + B13	0	0	0	11	11	22
Anolyte water + HWT	0	0	11	11	11	33
Anolyte water + B13	0	0	11	11	11	33
Anolyte water + HWT + B13	0	0	0	0	0	0
HWT + B13	0	0	0	0	0	0
Control	0	0	0	0	0	0

*Total percentage of decayed fruit at the end of the 28-day storage period. HWT, hot water treatment; +, 'combined with'.

The two-way interaction between the treatment and storage period had a significant influence on the decay severity as a result of *P. digitatum* at $P \leq 0.05$. As time progressed the decay caused by *P. digitatum* increased (Hong *et al*., 2007; Schirra *et al*., 2011). Hong *et al*. (2007) and Sen *et al*. (2007) attributed the reduction in decay in hot water treated citrus fruit to the melting and redistribution of natural epicuticular wax to seal cracks on the fruit surface. This creates a barrier for pathogen penetration. The reduction in decay could also be due to the host-pathogen interaction, where the combined effect of the pathogen and the hot water treatment induced resistance in the fruit peel. Hot water treatments also resulted in a reduction in the epiphytic microorganism population, which may prove to be beneficial (Hong *et al*., 2007). Biocontrol treatments have been found to be more effective in reducing decay when combined with other treatments such as hot water (Hong *et al*., 2014). The presence of B13 on the fruit surface colonises wounds by using up the nutrients produced by the wound (Abraham *et al*., 2012). Therefore, *Penicillium* spp. spores are unable to sporulate due to the lack of available nutrients. However, B13 is most effective when applied as a preventative treatment (Abraham *et al*., 2012).

The additional initial use of either chlorinated water or anolyte water as a disinfectant to remove some of the previously existing surface pathogens resulted in a lower decay severity. Furthermore, with the action of the hot water treatment to induce fruit resistance, as in the case of combined anolyte water, hot water and B13, no incidence of decay was observed due to the disinfecting action of the anolyte water, the curative action of the hot water and the preventative action of the B13. As mentioned in Section 4.5.2.1, anolyte water is described as having disinfecting properties and the B13 as having preventative properties. The addition of hot water further reduced the onset of decay due to the curative property that it is believed to possess (Ben-Yehoshua *et al*., 2005). Kim *et al*. (1991) observed an increase in scoparone in citrus fruit after inoculation with *P. digitatum*, which further increased after a heat treatment at 36°C for 3 days. The induced concentration of scoparone was sufficient to reduce fungal growth in lemon fruit. This also demonstrated the presence of pathogens to elicit fruit resistance. Lesar (2002) found that a dilution of anolyte water of 1:5 and 1:10 resulted in 100% spore eradication on citrus fruit with an exposure time ranging from 30 to 300 seconds. No visible decay of blue mould was observed on kumquat fruit for all treatments. This could be due to blue mould being more prevalent at cooler temperatures (<10°C) whereas at room temperatures (25°C) green mould develops at a faster rate (Brown, 1994). Schirra *et al*. (2011) also observed green mould to be the main decay agent in kumquat fruit. Therefore, the results obtained for the development of blue mould on the surface of the kumquat fruit has subsequently been omitted from this section. The results demonstrated that combined pre-packaging treatments proved to be more beneficial in inhibiting decay caused by green mould, compared to individual treatments. In particular the treatments of (1) anolyte water + hot water + B13 and (2) hot water + B13 were most beneficial in preventing decay caused by *P. digitatum* in kumquat fruit.

5.6.2 Physiological weight loss

Table 5.3 presents the physiological weight loss (PWL) of kumquat fruit as a result of *P. digitatum*. The treatment and storage period were found to have a highly significant ($P \leq 0.001$) effect on the physiological weight loss (PWL) of kumquat fruit. The four single treatments of (1) chlorinated water; (2) anolyte water; (3) hot water and (4) B13 resulted in higher PWL's of 86.17%; 77.76%; 71.81% and 81.14% on Day 28, respectively, compared to the combined treatments. Treatments including anolyte water as the

disinfectant produced lower PWL's, compared to fruit treated with chlorinated water as the disinfectant. Anolyte water in combination with hot water led to the lowest PWL of only 55.38% (Day 28). A large increase in the PWL can be observed between Days 14 and 21 and 21 and 28, particularly in the combined treatment of chlorinated water, hot water and biocontrol. The two-way interaction between the treatments and the storage period was found to be significant ($P \leq 0.05$) with regard to the PWL. Similarly, Singh and Reddy (2006) observed an increase in the cumulative weight loss of orange fruit with an increase in the storage period. The loss in weight could be attributed to (1) respiration where food reserves are used up and (2) transpiration where moisture is lost via microscopic cracks on the fruit surface (Hong *et al*., 2007).

Table 5.3 Changes in the physiological weight loss (%) of *Penicillium digitatum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0	**7**	**14**	**21**	**28**
Chlorinated water	0.00^{a}	34.72^{ef}	46.98^{hi}	60.04^{kl}	86.17^{p}
Anolyte water	0.00^{a}	26.94^{cd}	37.56^{efg}	58.16^{jkl}	77.76^{o}
Hot water	0.00^{a}	28.69^{cde}	42.67^{gh}	60.69^{kl}	71.81^{mno}
Biocontrol (B13)	0.00^{a}	34.34^{ef}	58.93^{jkl}	58.93^{jkl}	81.14^{op}
Chlorinated water + HWT	0.00^{a}	18.34^{b}	31.99^{de}	57.04^{jkl}	68.03^{m}
Chlorinated water + B13	0.00^{a}	21.95^{bc}	34.86^{ef}	60.05^{kl}	73.64^{no}
Chlorinated water + HWT + B13	0.00^{a}	28.11^{cde}	37.87^{efg}	49.48^{hij}	72.92^{mno}
Anolyte water + HWT	0.00^{a}	25.91^{bcd}	25.91^{bcd}	43.56^{ghi}	55.38^{jk}
Anolyte water + B13	0.00^{a}	29.44^{cde}	29.44^{cde}	59.96^{kl}	64.52^{lm}
Anolyte water + HWT + B13	0.00^{a}	23.7^{bc}	26.43^{cd}	47.32^{hi}	62.33^{lm}
HWT + B13	0.00^{a}	28.81^{cde}	35.85^{efg}	55.9^{jk}	64.1^{lm}
Control	0.00^{a}	31.67^{de}	43.07^{ghi}	53.7^{j}	70.4^{mn}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	*				
CV (%)	22.0				

NS, *, ** Non-significant or significant at $P \leq 0.05$ or $P \leq 0.001$, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

Table 5.4 shows the variation in the PWL values of kumquat fruit inoculated with *P. italicum* and subjected to different pre-packaging treatments. Similar trends were observed as in the case of green mould-inoculated fruit, where the PWL of the individual treatments were higher than combined treatments. Treatments that included anolyte water as the disinfectant resulted in lower PWL values than those treatments using chlorinated water. B13 alone resulted in the highest PWL of 77.25%, followed by 73.15% in fruit treated with chlorinated water + hot water + B13. The lowest PWL was observed in fruit treated with the combination of anolyte, hot water and B13 of 54.27%. Chlorinated water combined with hot water also resulted in a low PWL of 55.01% by Day 28. Similar observations of reduced weight loss was found by Hong *et al*. (2007) and Sen *et al*. (2007), which was attributed to melting of the epicuticular wax and sealing of surface cracks.

The two-way interaction between the treatments and the storage period had a slightly lower significance at $P \leq 0.05$, compared to the treatment and storage period. The increase in the PWL was highest toward the end of the storage period between Days 21 and 28. The increase in the weight loss could be attributed to the loss in moisture via the microscopic cracks, which appear on the fruit surface (Hong *et al*., 2007). The loss in weight can also be attributed to respiration where food reserves are being used up and transpiration where moisture is lost to the external environment. A high ambient temperature and low relative humidity further exacerbates these processes. The combined treatments proved to be better at reducing the PWL of kumquat fruit, compared to individual treatments. Treatments incorporating anolyte water reduced the PWL more than treatments using chlorinated water instead.

Table 5.4 Changes in the physiological weight loss (%) of *Penicillium italicum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0	**7**	**14**	**21**	**28**
Chlorinated water	0.00^{a}	21.10^{de}	34.95^{g}	46.9^{hij}	66.05^{l}
Anolyte water	0.00^{a}	16.83^{cd}	29.73^{efg}	50.42^{ij}	69.51^{lm}
Hot water	0.00^{a}	13.97^{bcd}	29.56^{efg}	38.11^{gh}	68.46^{lm}
Biocontrol (B13)	0.00^{a}	16.07^{cd}	35.73^{g}	41.3^{ghi}	77.25^{m}
Chlorinated water + HWT	0.00^{a}	9.91^{ab}	32.03^{fg}	35.18^{g}	55.01^{ijk}
Chlorinated water + B13	0.00^{a}	13.18^{bcd}	23.05^{def}	35.04^{g}	62.10^{kl}
Chlorinated water + HWT + B13	0.00^{a}	19.29^{cde}	27.76^{ef}	44.25^{hi}	73.15^{m}
Anolyte water + HWT	0.00^{a}	14.73^{bcd}	22.35^{de}	23.32^{def}	56.95^{jk}
Anolyte water + B13	0.00^{a}	9.42^{ab}	19.12^{cde}	44.07^{hi}	57.2^{jk}
Anolyte water + HWT + B13	0.00^{a}	17.24^{cd}	24.33^{def}	31.29^{efg}	54.27^{ijk}
HWT + B13	0.00^{a}	10.39^{abc}	21.35^{de}	23.22^{def}	56.48^{jk}
Control	0.00^{a}	11.06^{bc}	33.3^{fg}	35.36^{g}	70.07^{lm}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	*				
CV (%)	22.6				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

5.6.3 Peel colour

The changes in the hue angle of *P. digitatum*-inoculated kumquat fruit are presented in Table 5.5. The storage period had a highly significant (P≤0.001) influence on the hue angle, compared to the treatment, which was not found to be significant (P>0.05). The hue angle of each treatment was not significantly different per sample interval. However, the hue angle was observed to decrease from Day 0 to Day 28 for each treatment. Similarly, Smilanick *et al*. (2006) did not find a significant difference in the hue angle of treated and untreated citrus fruit. A decrease in the hue angle is indicative of a colour change from a yellow-lime to an orange-yellow.

Table 5.5 Changes in the hue angle (°) of *Penicillium digitatum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0	**7**	**14**	**21**	**28**
Chlorinated water	76.70^{h}	69.11^{g}	67.22^{def}	64.76^{cd}	58.91^{a}
Anolyte water	76.70^{h}	67.36^{def}	66.07^{de}	65.43^{cde}	64.77^{cd}
Hot water	76.70^{h}	66.83^{de}	66.54^{de}	64.70^{cd}	64.53^{cd}
Biocontrol (B13)	76.70^{h}	67.44^{def}	67.12^{de}	59.94^{b}	63.52^{bcd}
Chlorinated water + HWT	76.70^{h}	65.76^{cde}	66.82^{de}	65.35^{cde}	64.60^{cd}
Chlorinated water + B13	76.70^{h}	67.15^{de}	67.79^{def}	64.37^{cd}	64.20^{cd}
Chlorinated water + HWT + B13	76.70^{h}	67.93^{ef}	67.81^{def}	66.49^{de}	63.61^{bcd}
Anolyte water + HWT	76.70^{h}	68.77^{fg}	64.53^{cd}	65.26^{cde}	63.88^{bcd}
Anolyte water + B13	76.70^{h}	65.02^{cde}	66.28^{de}	64.17^{cd}	63.33^{bcd}
Anolyte water + HWT + B13	76.70^{h}	66.61^{de}	65.80^{cde}	64.19^{cd}	63.72^{bcd}
HWT + B13	76.70^{h}	70.34^{h}	66.94^{de}	65.87^{cde}	63.20^{bc}
Control	76.70^{h}	69.52^{gh}	65.11^{de}	63.65^{bcd}	62.47^{abc}
Significance					
Treatment (A)	NS				
Storage Period (B)	**				
AB	NS				
CV (%)	3.2				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

Chlorinated water and control samples caused the lowest hue angle of 58.91° and 62.47°. The change in the hue angle as a result of (1) anolyte water, (2) hot water, (3) chlorinated water + hot water and (4) chlorinated water + B13 were not significantly different on Day 28. The reduction in the hue angle occurred at a faster rate between Days 0 and 7, compared to later in the storage period where the hue angle remained fairly unchanged.

Table 5.6 shows the changes in the hue angle over a 28-day storage period of kumquat fruit inoculated with *Penicillium italicum* and subjected to different pre-packaging treatments. The storage period was found to be highly significant (P≤0.001) with regard to the hue angle. The reduction in the hue angle was most apparent between Days 0 and 7, thereafter remaining constant. A decrease in the hue angle can be indicative of ripening.

Therefore, the treatment which show the least decrease in the hue angle are the combined treatments, compared to individual treatments.

Table 5.6 Changes in the hue angle (°) of *Penicillium italicum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0	**7**	**14**	**21**	**28**
Chlorinated water	74.95^{g}	65.66^{cd}	66.16^{cd}	65.35^{cd}	65.29^{cd}
Anolyte water	74.95^{g}	66.92^{de}	66.66^{cde}	64.87^{bcd}	65.84^{cd}
Hot water	74.95^{g}	67.67^{def}	66.88^{de}	65.88^{cd}	65.01^{cd}
Biocontrol (B13)	74.95^{g}	67.05^{de}	65.52^{cd}	64.99^{cd}	65.69^{cd}
Chlorinated water + HWT	74.95^{g}	67.44^{de}	66.62^{cde}	65.88^{cd}	65.23^{cd}
Chlorinated water + B13	74.95^{g}	67.22^{de}	65.10^{cd}	65.72^{cd}	64.99^{cd}
Chlorinated water + HWT + B13	74.95^{g}	67.07^{de}	66.82^{de}	65.12^{cd}	63.52^{ab}
Anolyte water + HWT	74.95^{g}	66.41^{cd}	67.92^{ef}	65.91^{cd}	67.08^{de}
Anolyte water + B13	74.95^{g}	66.69^{cde}	66.21^{cd}	64.76^{bcd}	63.26^{a}
Anolyte water + HWT + B13	74.95^{g}	67.79^{ef}	66.97^{de}	64.88^{bcd}	64.50^{bc}
HWT + B13	74.95^{g}	68.20^{f}	66.25^{cd}	65.57^{cd}	64.21^{abc}
Control	74.95^{g}	66.65^{cde}	65.52^{cd}	65.18^{cd}	65.16^{cd}
Significance					
Treatment (A)	NS				
Storage Period (B)	**				
AB	NS				
CV (%)	1.7				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

5.6.4 Peel firmness

Table 5.7 presents the change in firmness of *P. digitatum*-inoculated kumquat fruit subjected to different pre-packaging treatments over a 28-day storage period. The treatments did not have a significant (P>0.05) influence on the peel firmness. A general decrease in the peel firmness can be observed from Day 0 to Day 28. However, a localized increase in firmness occurred, particularly in the control samples. A substantial increase in the firmness was observed between Days 14 (7.30 N) and 28 (9.04 N). Kumquats

treated with chlorinated water only displayed the least firmness (7.05 N), which is concomitant with the greatest PWL (86.17%) and lowest moisture content (40.53%), as indicated in Tables 5.2 and 5.9, respectively. Chlorinated water + B13 also displayed low fruit firmness on Day 28 of 7.10 N.

Table 5.7 Changes in the peel firmness (N) of *Penicillium digitatum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0	**7**	**14**	**21**	**28**
Chlorinated water	11.71^{e}	6.99^{a}	6.10^{a}	6.99^{a}	7.05^{a}
Anolyte water	11.71^{e}	10.60^{de}	8.08^{ab}	7.05^{a}	7.56^{ab}
Hot water	11.71^{e}	7.82^{ab}	7.57^{ab}	7.04^{a}	7.90^{ab}
Biocontrol (B13)	11.71^{e}	7.38^{ab}	7.41^{ab}	8.30^{ab}	8.48^{ab}
Chlorinated water + HWT	11.71^{e}	7.63^{ab}	7.98^{ab}	7.84^{ab}	7.20^{a}
Chlorinated water + B13	11.71^{e}	7.66^{ab}	7.90^{ab}	7.06^{a}	7.10^{a}
Chlorinated water + HWT + B13	11.71^{e}	7.36^{ab}	7.84^{ab}	7.54^{ab}	7.57^{ab}
Anolyte water + HWT	11.71^{e}	8.16^{ab}	9.07^{cde}	7.83^{ab}	7.68^{ab}
Anolyte water + B13	11.71^{e}	7.98^{ab}	8.90^{cde}	5.86^{a}	7.57^{ab}
Anolyte water + HWT + B13	11.71^{e}	8.71^{abc}	7.98^{ab}	8.51^{ab}	7.85^{ab}
HWT + B13	11.71^{e}	7.34^{ab}	7.88^{ab}	7.55^{ab}	7.82^{ab}
Control	11.71^{e}	8.12^{ab}	7.30^{a}	8.75^{bcd}	9.04^{cde}
Significance					
Treatment (A)	NS				
Storage Period (B)	**				
AB	NS				
CV (%)	18.5				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

Table 5.8 shows the variation in the firmness of kumquat fruit inoculated with *P. italicum* and subjected to different pre-packaging treatments. Unlike with *P. digitatum*-inoculated fruit, the treatment and storage period had a highly significant (P≤0.001) influence on the peel firmness. The control fruit exhibited the greatest increase in the firmness from Day 0 to Day 28 from 7.24 N to 12.76 N, amounting to a 76% increase in the firmness. The integration of chlorinated water and B13 also resulted in a large increase in the firmness

from Day 0 to Day 28 of 52%. This could largely be attributed to a reduction in the moisture content giving rise to a hard and leathery peel (Ladaniya, 2008).

Table 5.8 Changes in the peel firmness (N) of *Penicillium italicum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0	**7**	**14**	**21**	**28**
Chlorinated water	7.24^{ab}	7.47^{abc}	8.59^{cd}	9.18^{de}	9.36^{def}
Anolyte water	7.24^{ab}	7.04^{a}	7.25^{ab}	8.17^{bcd}	10.34^{gh}
Hot water	7.24^{ab}	7.17^{a}	7.18^{a}	7.38^{ab}	9.69^{efg}
Biocontrol (B13)	7.24^{ab}	7.65^{abc}	7.80^{bc}	8.31^{cd}	10.72^{hi}
Chlorinated water + HWT	7.24^{ab}	7.14^{a}	7.53^{abc}	7.83^{bc}	8.42^{cd}
Chlorinated water + B13	7.24^{ab}	7.67^{abc}	7.39^{ab}	8.66^{cd}	10.97^{j}
Chlorinated water + HWT + B13	7.24^{ab}	8.03^{bcd}	7.48^{abc}	8.46^{cd}	8.39^{cd}
Anolyte water + HWT	7.24^{ab}	7.86^{bc}	8.02^{bcd}	9.45^{ef}	9.96^{fg}
Anolyte water + B13	7.24^{ab}	8.15^{bcd}	8.21^{bcd}	8.56^{cd}	8.93^{cd}
Anolyte water + HWT + B13	7.24^{ab}	7.04^{a}	6.96^{a}	7.81^{bc}	8.02^{bcd}
HWT + B13	7.24^{ab}	7.64^{abc}	7.73^{bc}	8.48^{cd}	8.62^{cd}
Control	7.24^{ab}	7.41^{ab}	9.18^{de}	10.79^{hi}	12.76^{k}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	**				
CV (%)	10.3				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

The combined effect of anolyte water, hot water and B13 maintained the fruit firmness, which was evident in only an 11% increase in the fruit firmness. Chlorinated water combined with hot water and B13 as well as chlorinated water combined with hot water also demonstrated minimal variation in the fruit firmness of 16% throughout the storage period. Control fruit exhibited an increase in peel firmness associated with tissue lignification.

The one-way and two-way interaction between the treatment and storage period were highly significant (P≤0.001) on the firmness of kumquat fruit. The firmness in citrus fruit depends primarily on the turgidity and weight loss (Olmo *et al*., 2000; Hong *et al*., 2007). Olmo *et al*. (2000) found that a decrease in the firmness coincided with an increase in the weight loss. Studies by Rodov *et al*. (2000), Singh and Reddy (2006) and Hong *et al*. (2007) observed a decrease in the firmness of citrus fruit during storage. This was synonymous with a decrease in the moisture content resulting in a drying effect and softening of the peel tissue. However, Ladaniya (2008) observed that with increasing moisture loss, the peel of citrus fruit becomes tough and leathery, resulting in a higher puncture resistance. This could account for the increase in firmness, particularly between Days 21 and 28 in control fruit of *P. digitatum*- (9.04 N) and *P. italicum*-inoculated control fruit (12.76 N). The postharvest storage of fruit is associated in a loss in the cell wall integrity as a result of the breakdown of pectic substances (Valero *et al*., 1998). This in turn leads to an increase in the soluble pectin and a decrease in the fruit firmness. The combined treatment of a biocontrol agent (*Bacillus amyloliquefaciens* HF-01), hot water (45°C for 120 seconds) and sodium bicarbonate (1% or 2%) resulted in firmer mandarin fruit (Hong *et al*., 2014).

Many studies have found the combination of hot water and chlorinated water to be effective in extending the shelf life of citrus fruit (Korf *et al*., 2001; Sen *et al*., 2007). However, the addition of a biocontrol further improves the efficacy (Korf *et al*., 2001; Sen *et al*., 2007). This study found that the use of anolyte water as a disinfectant in integrated treatments was more beneficial in maintaining the fruit firmness than chlorinated water. Based on the results it can be recommended that the combined use of anolyte water, hot water and B13 biocontrol be used for the maintenance of the postharvest firmness of kumquat fruit.

5.6.5 Peel moisture content

Table 5.9 indicates the changes in the peel moisture content (MC) as a result of different pre-packaging treatments of kumquat fruit inoculated with *P. digitatum*. The treatment and storage period was highly significant (P≤0.001) with regard to the changes in the moisture content of the kumquat peel.

Table 5.9 Changes in the peel moisture content (%) of *Penicillium digitatum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0	**7**	**14**	**21**	**28**
Chlorinated water	82.80^{k}	71.9^{gh}	64.2^{de}	56.9^{bc}	40.53^{a}
Anolyte water	82.80^{k}	74.0^{hij}	63.6^{cde}	60.0^{cd}	50.1^{abc}
Hot water	82.80^{k}	66.0^{ef}	66.8^{ef}	59.1^{bcd}	50.1^{abc}
Biocontrol (B13)	82.80^{k}	72.6^{hi}	60.7^{cd}	59.5^{cd}	48.0^{ab}
Chlorinated water + HWT	82.80^{k}	70.3^{g}	67.7^{efg}	59.1^{bcd}	58.8^{bcd}
Chlorinated water + B13	82.80^{k}	75.0^{hij}	66.8^{ef}	58.2^{bcd}	50.3^{abc}
Chlorinated water + HWT + B13	82.80^{k}	68.5^{fg}	67.1^{ef}	61.2^{cde}	51.1^{abc}
Anolyte water + HWT	82.80^{k}	74.8^{hij}	64.6^{de}	63.9^{cde}	56.6^{bc}
Anolyte water + B13	82.80^{k}	70.4^{g}	63.1^{cde}	58.4^{bcd}	52.6^{abc}
Anolyte water + HWT + B13	82.80^{k}	66.0^{ef}	67.0^{ef}	62.9^{cde}	57.0^{bcd}
HWT + B13	82.80^{k}	71.2^{gh}	65.1^{def}	60.9^{cd}	51.7^{abc}
Control	82.80^{k}	68.3^{fg}	64.0^{de}	56.0^{bc}	42.37^{a}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	**				
CV (%)	5.3				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

A general decrease in the MC content was observed for all treatments from Day 0 to Day 28. The greatest decline in the MC was observed in control samples and samples treated with chlorinated water only of 49% and 51% from day 0 to day 28, respectively. The least decrease in the MC was observed in fruit treated with a combination of anolyte, hot water and biocontrol (31%) and chlorinated and hot water (29%) from Day 0 to Day 28. The two-way interaction between the treatment and the storage period was also found to be highly significant (P≤0.001) with regard to the peel moisture content. The rate at which the moisture content decreased was greater toward the end of the storage period, between Days 21 and 28. This was most pronounced after individual treatments, compared to integrated treatments. Treatments that combined anolyte water as the disinfectant had higher moisture contents than combined treatments including chlorinated water.

Table 5.10 represents the moisture content (MC) of the kumquat peel over a 28-day storage period that had been inoculated with *P. italicum*. The treatment and storage was found to significantly ($P \leq 0.001$) influence the MC. A gradual decrease in the moisture content was observed in all treatments throughout the storage period. The individual treatments resulted in kumquat fruit with lower MC than those subjected to integrated treatments. The combined treatments of (1) chlorinated water and hot water, (2) anolyte water, hot water and B13 and (2) hot water alone produced the highest MC of 56.3%, 54.6% and 54.6%, respectively, on Day 28. The individual treatments resulted in the lowest MCs on Day 28, compared to combined treatments. The two-way interaction between the treatments and storage periods was highly significant ($P \leq 0.001$) in terms of the peel moisture content. The greatest reduction in the MC was observed at the end of the storage period between days 21 and 28.

Citrus fruit have a high moisture content in both the pulp and peel (Chien *et al*., 2007; Ghanema *et al*., 2012). Once harvested the fruit loses excessive moisture from the peel via transpiration and respiration, promoting the onset of decay caused by pathogens, thereby reducing the shelf life (Purvis, 1983). Treatments incorporating anolyte water as the disinfectant were more effective in maintain the fruit MC.

Table 5.10 Changes in the peel moisture content (%) of *Penicillium italicum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0	**7**	**14**	**21**	**28**
Chlorinated water	80.4^{f}	70.2^{cd}	63.5^{bc}	53.1^{ab}	46.5^{a}
Anolyte water	80.4^{f}	70.8^{cde}	66.6^{bcd}	56.8^{abc}	40.0^{a}
Hot water	80.4^{f}	74.7^{ef}	69.0^{cd}	61.3^{bc}	54.6^{ab}
Biocontrol (B13)	80.4^{f}	70.3^{cd}	64.4^{bc}	57.1^{abc}	43.7^{a}
Chlorinated water + HWT	80.4^{f}	73.4^{def}	68.7^{cd}	64.6^{bc}	56.3^{abc}
Chlorinated water + B13	80.4^{f}	71.8^{de}	67.5^{bcd}	61.2^{bc}	46.4^{a}
Chlorinated water + HWT + B13	80.4^{f}	71.0^{cde}	68.9^{cd}	58.3^{abc}	50.5^{ab}
Anolyte water + HWT	80.4^{f}	72.4^{de}	62.8^{bc}	62.7^{bc}	53.0^{ab}
Anolyte water + B13	80.4^{f}	70.9^{cde}	65.7^{bcd}	56.3^{abc}	44.9^{a}
Anolyte water + HWT + B13	80.4^{f}	72.3^{de}	68.9^{cd}	63.3^{bc}	54.6^{ab}
HWT + B13	80.4^{f}	70.5^{cde}	67.0^{bcd}	67.3^{bcd}	52.2^{ab}
Control	80.4^{f}	71.7^{de}	67.1^{bcd}	57.8^{abc}	50.8^{ab}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	**				
CV (%)	5.4				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

5.6.6 Total soluble solids

The changes in the total soluble solids (TSS) of kumquat fruit inoculated with *P. digitatum* and subjected to different pre-packaging treatments are presented in Table 5.11. The treatments were not found to be significant (P>0.05). However, the storage period was found to be highly significant (P≤0.001) with regard to the changes in the TSS. Chlorinated water and control samples exhibited substantial increases in the TSS from Day 0 to Day 28 of 82% and 75%, respectively. Comparatively, anolyte water combined with hot water and biocontrol resulted in the least increase in the TSS of 54% over the 28 days of storage. Similarly, chlorinated water and hot water resulted in a 55% decrease in the TSS. The rate of increase in TSS occurred at a faster rate at the start of the storage

period from Day 0 to Day 14, compared to days 14 to 28. The TSS of kumquats for individual treatments and the control were higher, compared to those that were exposed to integrated pre-packaging treatments.

Table 5.11 Changes in the total soluble solids (°Brix) of *Penicillium digitatum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0	**7**	**14**	**21**	**28**
Chlorinated water	10.2^{a}	12.0^{ab}	14.9^{cd}	16.0^{def}	18.5^{hi}
Anolyte water	10.2^{a}	12.4^{ab}	14.3^{bcd}	15.3^{cd}	16.3^{ef}
Hot water	10.2^{a}	13.4^{abc}	14.0^{bc}	15.6^{cde}	16.3^{ef}
Biocontrol (B13)	10.2^{a}	12.0^{ab}	15.6^{cde}	15.9^{de}	16.4^{efg}
Chlorinated water + HWT	10.2^{a}	13.5^{abc}	14.5^{bcd}	16.8^{gh}	15.8^{de}
Chlorinated water + B13	10.2^{a}	12.2^{ab}	13.5^{abc}	16.4^{ef}	16.7^{fg}
Chlorinated water + HWT + B13	10.2^{a}	12.9^{abc}	13.35^{abc}	16.5^{efg}	16.7^{fg}
Anolyte water + HWT	10.2^{a}	12.1^{ab}	15.1^{cd}	16.0^{def}	16.0^{def}
Anolyte water + B13	10.2^{a}	10.8^{a}	14.3^{bcd}	16.1^{def}	15.8^{de}
Anolyte water + HWT + B13	10.2^{a}	9.93^{a}	14.2^{bcd}	15.2^{cd}	15.7^{cde}
HWT + B13	10.2^{a}	11.6^{ab}	13.1^{abc}	15.2^{cd}	16.6^{efg}
Control	10.2^{a}	11.9^{ab}	15.6^{cde}	16.1^{ef}	17.8^{h}
Significance					
Treatment (A)	NS				
Storage Period (B)	**				
AB	NS				
CV (%)	9.3				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

Table 5.12 depicts the changes in the TSS of *P. italicum*-inoculated kumquat fruit subjected to different pre-packaging treatments for a 28-day storage period. The treatment and storage period were found to have a highly significant (P≤0.001) effect on the TSS of kumquat fruit. A general increase in the TSS was observed for kumquat fruit under all treatments. Kumquat fruit treated with chlorinated water only and control samples were observed to have highest TSS values of 18.2 °Brix and 18.5°Brix, respectively.

The two-way interaction between treatment and storage period was found to be highly significant (P≤0.001) with regard to the TSS of kumquat fruit. The least increase in the TSS was found in samples treated with the combination of anolyte water, hot water and biocontrol of 14.8 °Brix. The TSS was found to increase more rapidly at the start of the storage period between Days 0 and 7.

Table 5.12 Changes in the total soluble solids (°Brix) of *Penicillium italicum*-inoculated kumquat fruit over a 28-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0	**7**	**14**	**21**	**28**
Chlorinated water	10.7^{a}	13.5^{cd}	14.9^{de}	15.6^{ef}	18.2^{h}
Anolyte water	10.7^{a}	13.1^{bcd}	13.5^{cd}	15.1^{def}	15.9^{ef}
Hot water	10.7^{a}	11.7^{ab}	13.4^{bcd}	15.4^{def}	15.7^{ef}
Biocontrol (B13)	10.7^{a}	12.8^{bc}	14.5^{cde}	15.3^{def}	17.3^{g}
Chlorinated water + HWT	10.7^{a}	12.8^{bc}	13.5^{cd}	14.5^{cde}	17.3^{g}
Chlorinated water + B13	10.7^{a}	13.7^{cd}	13.8^{cd}	14.5^{cde}	16.0^{ef}
Chlorinated water + HWT + B13	10.7^{a}	12.8^{bc}	13.4^{bcd}	14.9^{de}	16.3^{efg}
Anolyte water + HWT	10.7^{a}	13.0^{bcd}	13.3^{bcd}	13.5^{cd}	16.3^{efg}
Anolyte water + B13	10.7^{a}	13.1^{bcd}	14.0^{cde}	15.0^{def}	15.4^{def}
Anolyte water + HWT + B13	10.7^{a}	12.7^{bc}	13.1^{bcd}	14.6^{de}	14.8^{de}
HWT + B13	10.7^{a}	12.7^{bc}	13.8^{cd}	15.2^{def}	16.7^{fg}
Control	10.7^{a}	12.8^{bc}	14.4^{cde}	15.4^{def}	18.5^{h}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	**				
CV (%)	5.6				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; +, 'combined with'.

An increase in the TSS of citrus fruit have been observed by D'hallewin *et al*. (1994), Olmo *et al*. (2000), Rodov *et al*. (2000) and Ladaniya (2008), which can be attributed to a loss in water after harvest. Therefore, as the fruit matures an increase in the TSS is expected. However; Hong *et al*. (2007) found that the TSS decreased in Satsuma mandarin, which could be attributed to the catabolism of sugars and organic acids for

plant tissue metabolism. In addition, the degradation of cellulose, hemicellulose and pectin from the cell walls of the fruit segments may release soluble components, which directly increases the TSS (Roongruangsri *et al*., 2013). D'hallewin *et al*. (1994) found that the TSS in heat-treated (36°C for 72 hours) and UV-treated (24 nm) Avana mandarins were lower, compared to control samples at 7.85, 7.63 and 8.02 °Brix, respectively. Hong *et al*. (2014) found that the combined treatment of hot water, biocontrol and sodium bicarbonate resulted in mandarin fruit with lower TSS values, compared to control samples.

Based on the results it can be stated that the use of integrated treatments are beneficial in reducing the rate of increase of the TSS, which is an indication of a slower maturation rate. The use of anolyte water, hot water and biocontrol have been found to be the most effective treatment in reducing the rate of maturation of kumquat fruit.

5.7 Conclusion

This study investigated the effects of chlorinated water, anolyte water, hot water and a biocontrol agent, B13 (a strain of *C. fermentati*) applied as pre-packaging treatments on the quality of kumquat fruit. The study revealed that integrated pre-packaging treatments were more effective at reducing the onset of decay caused by *P. digitatum*, compared to individual treatments on kumquat fruit. The use of anolyte water (disinfectant) removes the surface pathogens, the hot water treatment (preventative) has the potential to seal surface wounds and initiates the fruit resistance to defend itself, while the biocontrol agent (preventative) has the ability to defend the fruit against future infection by colonizing wounds. Therefore, it can be deduced that treatments including a disinfectant combined with preventative and curative treatments have the best potential to protect fruit and ensure better quality over a longer period of time.

The application of anolyte water as a disinfectant caused better results in terms of decay severity, PWL, firmness, MC, and TSS than chlorinated water. Anolyte water combined with hot water resulted in firmer fruit with higher MC values. However, chlorinated water combined with hot water produced similar results to that of anolyte water, hot water and biocontrol in terms of the MC and TSS. The use of anolyte water, hot water and biocontrol had a beneficial effect on decay severity, PWL, firmness, MC and the TSS, which were

similar to those of (1) anolyte water combined with hot water and (2) chlorinated water combined with hot water. The decay severity, PWL, firmness, peel MC and TSS were 0%, 62.33%, 7.85 N, 57.0% and 15.7, respectively, by Day 28. Therefore, it can be deduced that treatments including a surface disinfectant (anolyte water), hot water and the B13 biocontrol agent were effective in maintaining desirable fruit quality. These treatments were then incorporated in the design of the prototype treatment unit presented in Chapter 6. This research was aimed at small-scale farmers, who are able to adopt this form of technology without the reliance on commercial packhouses. It is envisaged that this equipment can be transported directly to the orchard to treat fruit before transport to packhouses, thereby reducing decay.

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6. DEVELOPMENT AND EVALUATION OF A SMALL-SCALE IN-FIELD INTEGRATED POSTHARVEST CITRUS TREATMENT UNIT

6.1 Abstract

Exposure of kumquat fruit to high ambient temperatures during transport to packhouses leads to decay and excessive deterioration. Therefore, the aim of this study was to develop an integrated postharvest citrus treatment unit (IPCTU) capable of being operated on site at the orchard. The IPCTU consists of five systems including a rinsing tank (RT), surface disinfection tank using anolyte water (AT), a hot water tank (HWT), a drying zone consisting of a conveyor belt and fans (DZ) and a biocontrol tank (BT). The IPCTU was mainly constructed from grade 304 stainless steel with a temperature control unit and a circulation pump on the HWT and BT systems. The drying section was composed of a conveyor belt and hand crank. An energy analysis revealed that 4.13 kW and 2.08 kW of electricity was consumed by the HWT and BT because these tanks required heating. The thermal efficiencies of HWT and BT were 72% and 87%, respectively. The total carbon ratio for the prototype IPCTU was 0.46 kg $CO_2.day^{-1}$ per operating period. The payback period for the IPCTU on a commercial scale was found to be 0.91 years. An exposure time of (1) anolyte water × 30s + hot water × 30s × 60°C + B13, (2)) anolyte water × 30s + hot water × 20s × 60°C + B13, (3) anolyte water × 30s + hot water × 30s × 53°C + B13 proved to be successful in eliminating decay.

6.2 Introduction

The South Africa kumquat season starts in May and ends in October. Once harvested, the fruit are transported in non-refrigerated vehicles with other fruit and vegetable to packaging houses, which are located away from the point of harvest. This is undesirable because the fruit are untreated and subjected to high field temperatures during transportation. Rapid deterioration of horticultural crops can be directly attributed to field heat due to the high temperatures, promoting increased rates of respiration and metabolism (Brosnan and Sun, 2001). Therefore, the removal of field heat can be described as the most important operation required to maintain the desirability, freshness

and salability of fruit and vegetable immediately after harvest in field (Brosnan and Sun, 2001). In addition, kumquats have relatively low yields, compared to other citrus, and requires transportation of fruit consignments from several farmers or with other crops. If kumquats are transported with incompatible crops, this can result in increased respiration rates (TransFresh Corporation, 1999).

Penicillium digitatum and *P. italicum* are the most common postharvest pathogens affecting kumquat fruit (Schirra *et al*., 2011; Youssef *et al*., 2014). The adverse influence of the field heat through the delayed transporting of the untreated fruit combined with the *Penicillium* pathogens can result in severe crop losses. This drives the development of novel postharvest handling techniques. Fungicides such as sodium orthophenylphenate (SOPP) and/or thiabendazole (TBZ) have been used to control postharvest decay of kumquats (Hall, 1986). However, the dependence on fungicides results in resistant strains of key pathogens. There are also concerns for potential toxic effects by consumers. More consumer and environmentally friendly treatments are required such as anolyte water (Lesar, 2002; Workneh *et al*., 2003; Workneh *et al*., 2011), hot water dipping (Sen *et al*., 2007; Hong *et al*., 2014), and biocontrol agents (Abraham *et al*., 2010). Combining these treatments may provide for a highly effective treatment to alleviate the onset of postharvest decay of kumquat (Sen *et al*., 2007; Hong *et al*., 2014). To address the challenge of transportation and field heat, specialised equipment is needed to treat fruit in the orchard immediately after harvest, an approach which has not been previously been documented. Numerous studies have concentrated on the integrated effect of treatments on orange, grapefruit and lemon. However, limited research, particularly in South Africa, has delved into the integrated effects of these combined treatments on kumquats (Choi, 2005; Sisquella *et al*., 2013). This necessitated research into the integrated effect of treatments to manage the processing of kumquat fruit before they are transported to packhouses. These techniques have the potential to enhance fruit quality all the way from the field to the final market destination.

This study was aimed at developing an integrated postharvest citrus treatment unit (IPCTU) to be used by small-scale farmers at the orchard. The IPCTU must incorporate the effective treatments from Chapter 5 to reduce decay of kumquat fruit caused by *P. digitatum*.

The specific objectives formulated for this study were to:

1. Develop a unit with multiple pre-packaging treatments.
2. Evaluate the overall efficiency of the pre-packaging treatment unit in terms of power and water use.
3. Perform a cost benefit analysis of the integrated pre-packaging treatment unit.
4. Determine the effect on the overall quality of the sample fruit.

The research questions that emerged from this study were as follows:

1. How can the integrated postharvest citrus treatment unit (IPCTU) be efficiently designed to incorporate the anolyte water, hot water and B13 treatments?
2. How can the IPCTU be designed to be mobile and operational at the orchard.

Both *P. digitatum* and *P. italicum* inoculants were used in Chapter 5. However, *P. digitatum* was more prevalent, compared to *P. italicum.* Therefore, only *P. digitatum* was used to inoculate the sample kumquat fruit in this study.

6.3 Design Considerations and Description of the IPCTU

The IPCTU capacity was targeted at 100 kg of kumquat which can be processed per treatment session. This capacity was sufficient for sampling purposes before being converted to a commercial scale. Each treatment batch was 10 kg based on the size of a picker bag of 2 kg (equivalent to 5 pickers). Therefore, a total of 10 batches could be done per day. The results obtained from Chapter 5 were used to determine the most suitable combination of pre-packaging treatments for kumquat fruit (Kassim *et al*., 2016). All surfaces in contact with the fruit were manufactured from 0.9 mm thick 304 stainless steel (food grade). The footprint of the IPCTU was confined to the dimensions of a 20-foot dry container (6 m × 2.4 m × 2.2 m), which will be used to house the IPCTU. This was important as the use of a dry container would facilitate the mobility of the IPCTU and protect it from exposure to the environment. The sizing of each zone was based on the physical properties obtained from Chapter 3.

The IPCTU comprised of six processing zones and operates as follows:

1. Zone 1: Initial rinsing where fruit are rinsed by hand in a tank of potable water.
2. Zone 2: Secondary rinsing. The amount of rinsing depends on the amount of field dirt present on the fruit surface. A thorough rinsing to remove dirt from the fruit

is essential to maintain the efficacy of the anolyte water. Fruit are then emptied into a perforated trapezoidal trough attached to a 400 kg double line electric hoist on a manual trolley to allow for horizontal and vertical movement between treatment steps. The perforations in the trough permit excess liquid to drain out before fruit is immersed in the next treatment tank.

3. Zone 3: Anolyte water (Main Treatment 1). The trough is dipped into a tank containing anolyte water at ambient conditions for a specified time. The trough is then raised to drain excess anolyte water to drip out before the next treatment.
4. Zone 4: Hot water dipping (Main Treatment 2). The trough is then lowered into a tank containing the hot water at the required temperature and time.
5. Zone 5: Removal of surface moisture after draining free water. The fruit is emptied onto a food grade PVC conveyor belt, which is manually operated. The surface moisture is removed with the aid of two propeller fans situated overhead with adjustable angles of inclination. Removal of surface moisture is necessary prior to exposure to the biocontrol agent to allow for adherence to the fruit surface. Removal of the surface moisture also removes excess heat from the fruit so as to return to ambient temperature (Fallik, 2004).
6. Zone 6: B13 biocontrol agent (Main Treatment 3). The fruit is then directed into a perforated trough, which is lowered into a tank containing the biocontrol agent.

Upon completion of the treatment the fruit are air dried at ambient conditions to allow the biocontrol agent to adhere to the surface.

The flexibility of the system allows the user to change the order or even omit treatments depending on the fruit due to each zone being separate. The IPCTU was designed based on the results from previous Chapters 3 (fruit physical properties), 4 and 5 (effective treatments). The tank bases are inclined at 80° to allow for easy drainage of liquid via an outlet tap. Each tank is supported by a galvanised steel stand. The sheets of steel were spot welded and the joints were sealed with silicone to prevent leaks. Both the hot water tank and the biocontrol tanks are fitted with a 2 kW heating element and a temperature control system to adjust each tank to the required temperature. These tanks were also fitted with household washing machine pumps and 24-hour universal manual timers. The pumps facilitated water circulation to allow for even heating of water. The timers ensure that the pumps were not continuously in operation to reduce power consumption and to avoid mechanical damage or pump failure. Due to the high temperatures required for the

hot water tank, compared to the biocontrol tank, a 50 mm thick foil-faced glasswool geyser blanket was used as insulation. The insulation was fitted along the four sides of the tank. Figure 6.1 illustrates the top and front views of the system including the dimensions of each of the six zones. Figure 10.1 in Appendix B shows different components of the treatment unit.

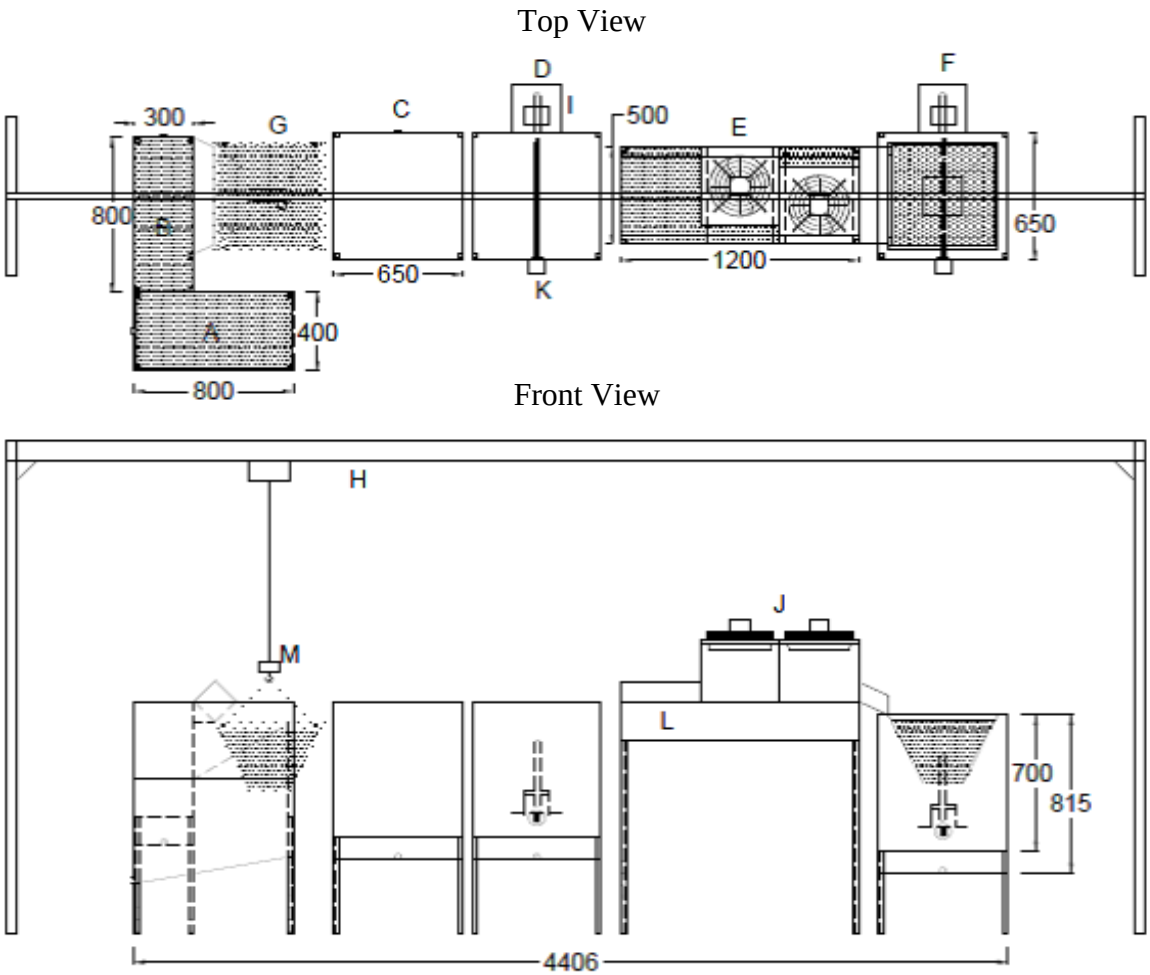

Figure 6.1 Schematic diagram of the IPCTU: A, rinse tank 1; B, rinse tank 2; C, anolyte water tank; D, hot water tank; E, surface moisture removal zone; F, biocontrol tank; G, perforated trough; H, gantry; I, circulation pump; J, two propeller fans; K, heating element; L, conveyor belt, M, electric hoist on manual trolley

6.4 Materials and Methods

6.4.1 Sample fruit production

Nagami (*Fortunella margarita*) was identified as the sample fruit. The same procedure was followed as per Section 4.3.1. A total of 15 kg of kumquat fruit were used for this experiment.

6.4.2 Thermal efficiency and energy analysis

The temperatures at nine different points of the three main tanks (anolyte water, hot water, biocontrol) and the ambient conditions were recorded using a CR10X data logger (Campbell Scientific Inc., Cape Town, South Africa) connected to a 12 volt battery and E-type thermocouples (chromel constantan). The temperature was measured at the following points:

1. Approximately 30 mm below the surface of the liquid in Tank C.
2. Approximately 30 mm below the surface of the liquid in Tank D and F.
3. Approximately 30 mm below the surface of the liquid in Tank F.
4. 700 mm from the top of Tank C.
5. 700 mm from the top of Tank D.
6. 700 mm from the top of Tank F.
7. 350 mm from the top of Tank D between the insulation and tank.
8. 350 mm from the top of Tank D on the inside.
9. The prevailing ambient temperature.

An Ellies wireless electricity monitor (Ellies, Johannesburg, South Africa) for single-phase current was used to record the energy consumed by the IPCTU. The electricity monitor was set up to measure the energy consumed (kW or $kW.h^{-1}$), cost per day ($R.day^{-1}$) and carbon footprint (kg $CO_2.day^{-1}$) for the duration of operation of the IPCTU. Elink 2.2 Energy Management software was used to interpret the energy consumption in conjunction with the electricity monitor. Equations 6.1 and 6.2 describe the heat loss from the system without insulation and with insulation, respectively (ASHRAE, 1989; Carpenter and Kissock, 2006). The efficiency of the system was determined using

Equation 6.3. Based on these equations the energy saving can be calculated using Equation 6.4.

$$Q_{uninsulated} = h \times A \times (T_s - T_a) + \sigma \times A \times \varepsilon \times (T_s^4 - T_a^4) \quad (6.1)$$

where:

Q = heat loss [J],

h = convection coefficient,

A = area of heat exchange [m^2],

T_s = surface temperature [°C],

T_a = surrounding temperature [°C],

σ = Stefan-Boltzman constant (5.6697×10^{-8} $w.m.^{-2}.k^{-4}$), and

ε = emissivity of material (0.0-0.9).

$$Q_{insulated} = A \times (T_f - T_i) / (R_c + R_i) \quad (6.2)$$

where:

T_f = temperature of fluid inside tank [°C],

Ti = outside temperature of insulation [°C],

R_c = thermal resistance of stainless steel [m^2.K/W], and

R_i = thermal resistance of insulation [m^2.K/W].

$$\eta = [m \times c \times (T_2 - T_1)]/(P \times t) \quad (6.3)$$

where:

η = thermal efficiency,

m = mass of fluid [kg],

c = specific heat of fluid (4.18 $J.g^{-1}°C^{-1}$),

T_2 = final temperature [°C],

T_1 = initial temperature [°C],

P = power [W], and

t = time [s].

$$E_{savings} = (Q_{uninsulated} - Q_{insulated})/ \eta \quad (6.4)$$

6.4.3 Pre-packaging treatments

The experiments were full factorial and performed in triplicate with three replications on kumquat fruit inoculated with *P. digitatum*. Fruit were inoculated prior to the application of the treatments. Fruit were treated with a combination of treatments comprising:

1. Two disinfection times (30 and 60 seconds) with anolyte water.
2. Two hot water temperatures (53°C and 60°C).
3. Two hot water dipping times (20 and 30 seconds).
4. *Candida fermentati* yeast isolate – B13 (biocontrol agent) according to the supplier's recommendations (27°C for 10 seconds).
5. Control samples were dipped in potable water at ambient temperature (26°C).

6.4.4 Experimental Design

The experiments were full factorial and performed in triplicate with three replications This was conducted on kumquat fruit inoculated with *Penicillium digitatum* only. Fruit were inoculated prior to the application of the treatments. The experimental design is contained within Appendix B – Table 10.1. A total of 36 different combination treatments were applied to *P. digitatum*-inoculated kumquat fruit. After treatment, the fruit were stored at ambient conditions for 14 days at 23°C and 66% relative humidity, as recorded by the data logger. Fruit were sampled on Days 0, 7 and 14.

6.4.5 Isolation of *Penicillium digitatum* from infected fruit

The method explained in Chapter 5, Section 5.3.4 for the isolation of *P. digitatum* was followed.

6.4.6 Sample preparation

The same procedure as mentioned in Section 4.3.5 was used. The fruit were then sorted into 36 batches of 27 fruit each and labelled at the base of the fruit using a white marker.

6.4.7 Inoculation of kumquat using *Penicillium digitatum*

P. digitatum conidia that had been prepared as explained in Section 6.4.5 was used. The method explained in Chapter 5, Section 5.3.6 for the inoculation of *P. digitatum* was followed.

6.5 Data Collection and Analysis

The effect of the treatments on the kumquat fruit were evaluated based on the change in the physical and microbiological quality of the fruit. The physical quality parameters that were investigated were the physiological weight loss and the peel firmness. The microbiological quality parameter was based on the decay severity as a result of *P. digitatum*.

6.5.1 Decay severity

Decay severity was evaluated based on the measured dimensions and calculated surface area that had fungal development and expressed as a percentage of the entire surface area. The dimensions were measured using a digital Mitutoyo Vernier calliper (Mitutoyo Corporation, Kanawa, Japan) (Hong *et al*., 2007; Abraham *et al*., 2010; Schirra *et al*., 2011).

6.5.2 Physiological weight loss

Kumquat fruit were individually weighed using a Mettler PJ 300 scale (Mettler-Toledo, Barcelona, Spain) at the start of the experiment and at the specified sampling intervals of 7 days. The differential weight loss was calculated for each sample per interval and converted to a percentage of the original fresh weight of the fruit (wet basis) (Singh and Reddy, 2006; Hong *et al*., 2007).

6.5.3 Peel firmness

An Instron Universal Testing Machine (Model 3345) (Advanced Laboratory Solutions, Baar, Switzerland) was used in conjunction with the Instron Bluehill 2 Version 2.25

software to determine the firmness of the kumquat peel by means of puncturing the fruit surface. Individual unpeeled kumquat fruit were placed horizontally on the curved platform (stem axis parallel to plate). A probe of 1.5 mm diameter was used to perform two punctures per fruit sample on opposite sides of the equatorial region. The cross head speed was set at 200 $mm.min^{-1}$ to travel to a depth of 12 mm. The maximum force required to puncture the fruit was taken as the exterior fruit firmness (Valero *et al*., 1998).

6.6 Economic evaluation

The payback period can be explained as the period of time required for a project to make a net profit based on Equation 6.5 (Tilahun, 2010; Wang *et al*., 2015). The costs involved in the economic analysis are the fixed and variable costs. Fixed costs comprise once-off payments for construction materials and labour. Variable costs include electricity, water, labour for operation and consumables.

$$PP = C/NE \tag{6.5}$$

where:

PP = Payback period,

C = Cost [R], and

NE = Net earnings [R]

6.7 Statistical Data Analysis

The statistical analysis of the decay severity, physiological weight loss and peel firmness was performed by the GenStat software, 17th Edition. The differences between treatments were determined by an analysis of variance (ANOVA) and the means were separated using the Duncan's Multiple Range Test, with a significance level of 0.05.

6.8 Results and Discussion

6.8.1 Thermal efficiency and energy analysis

The total power consumption for Tank D and Tank F was 4.13 kW and 2.08 kW, respectively. The heat loss with insulation for Tank D was determined to be 41.82 J, while

omitting insulation resulted in a heat loss of 285.31 J. The importance of proper design can, therefore, be demonstrated because 243.50 J of heat was retained in the system due to insulation, which also has a cost saving factor. Due to the lower temperature of Tank F (27°C), insulation was not necessary as an analysis revealed that only 15.02 J of energy would be retained in the system. It was noted that more heat was lost as a result of convection, compared to radiation in both insulated and uninsulated cases. Therefore, greater emphasis should be placed on reducing heat loss through convection with regard to the prototype. Water temperatures recorded in the upper portion of the tanks were higher, compared to temperatures lower down. This can be attributed to thermal stratification (Knudsen and Furbo, 2004). Circulation pumps were, therefore, used to ensure uniform heating.

The thermal efficiency of Tank D was found to be 72% in heating approximately 214 litres of water from an initial temperature of 22.54°C to 58.13°C. While Tank F was found to be 87% efficient in raising the temperature from 22.68°C to 26.32°C of the biocontrol media. On a commercial scale these values would increase considerably. Therefore, all measures must be taken to ensure that as much heat as possible is retained in the system by employing adequate insulation. Energy saving is an important aspect because this has a direct economic effect. One of the methods of reducing heat loss so as to increase the system efficiency would be to use and install suitable construction materials with adequate thermal and durability properties. Another method would be in the operation of the IPCTU. By reducing the operation and fruit treatment time, the efficiency of the IPCTU can also be increased with reduced heat loss. The temperature profiles of Tanks C, D and F are illustrated in Figure 6.2.

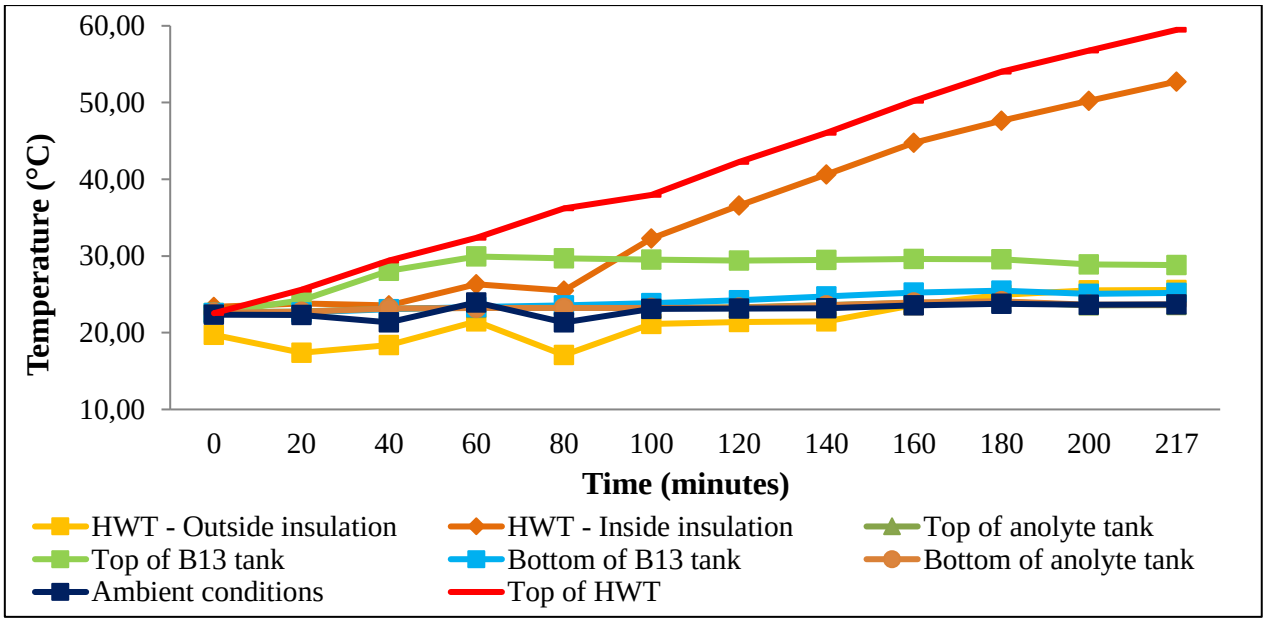

Figure 6.2 Temperature profile at various locations of the anolyte water tank – Tank C; hot water tank – Tank D and B13 tank –Tank F

The mean temperatures calculated for the top and bottom of Tank C was 22.36°C and 22.37°C, respectively, indicating an insignificant variation in the tank temperature. The overlapping of these graphs can also be seen in Figure 6.2. The mean outside temperature was 23.04°C, which is similar to the mean temperature of Tank C. This was expected because the tank did not have any insulation installed and, therefore, was exposed to the ambient conditions. The mean temperatures at the top and bottom of Tank F was 28.55°C and 24.08°C, a variation of 4.47°C. The top of the tank recorded a lower temperature due to the surface of the solution being exposed to ambient conditions. Therefore, energy was lost through evaporation (latent heat of vaporization). Temperatures between 25°C and 28°C are recommended for the B13 yeast to be effective. A time of approximately 217 minutes was required for the Tank D to reach the required temperature. Insulation is essential in reducing the time required for the optimal temperature to be reached as less heat energy is lost to the surroundings.

The carbon ratio is also an essential factor to consider, South Africa being one of the largest CO_2 emitting countries in Africa (Asane-Otoo, 2015). The carbon ratio for the prototype was measured to be only 0.46 kg $CO_2.day^{-1}$. Low carbon ratios are desirable as South Africa contributes to more than 30% of Africa's respective production- and consumption-based emissions. In referring to studies regarding the design of similar

equipment, it was observed that many neglected to determine the carbon ratio of the equipment.

6.8.2 Economic evaluation

The mean kumquat production achieved for South Africa for the 2013/2014 and 2014/2015 kumquat seasons was 83 000 kg. The price per kg of kumquats was R 25.00, amounting to an annual income of R2 075 000.00, assuming 100% marketability. The payback period and itemization of the cost components for the fixed and variable costs of the IPCTU are presented in Table 6.1. The values obtained and calculations were based on the following assumptions:

1. The IPCTU will be processing 100 kg per treatment period throughout the 6 month harvesting season each year.
2. The electricity tariff of 150 $c.kW^{-1}.h^{-1}$ as at July 2016 (Revocation and Determination of Tariffs for the 2016/2017 Financial Year, 2016).
3. The cost needs to, therefore, consider the different tariffs depending on the month of operation.
4. The time for one complete treatment operation is 4 hours.
5. Water tariff of R 21.91 per kL as at July 2016 (Revocation and Determination of Tariffs for the 2016/2017 Financial Year, 2016).
6. Values for the electricity and water tariff are for the Limpopo Province, since the bulk of the kumquat producers are located in that area.
7. Unskilled labour rate of R 2 778.83 per person per month as at March 2016 (Basic Conditions of Employment Act, 2016).
8. 10 persons are employed.
9. Consumables include purchasing the B13 biocontrol agent and the anolyte water.

Table 6.1 Summary of the economic evaluation of the IPCTU

Annual Income	**Fixed Cost**		**Variable Cost**				**Payback Period (Year)**
	Construction Material	**Labour for Construction**	**Electricity**	**Water**	**Labour**	**Consumables**	
R2 075 000	R35 849	R15 000	R48 274	R10 104	R166 730	R1 616 548	0.91
152 366 USD	2 632 USD	1 101 USD	3 545 USD	742 USD	12 243 USD	1 18 702 USD	0.91

The payback period for the IPCTU was calculated to be 0.91 years. De Oliveira *et al*. (2016) calculated a payback period of 7 years for a dryer column with a steam system in place of the conventional furnace for grain drying. Other postharvest equipment such as a drier for logan fruit was found to have a payback period of less than 3 years (Tippayawong *et al*., 2008). Therefore, it can be deduced that a payback period of 0.91 years is economically viable. The economic advantage of this equipment safeguards against the reliance on packhouses by small-scale farmers, promoting self-reliance. In addition, employment opportunities are created during the IPCTU's operation without the requirement of specialised skills. The materials used for construction were locally sourced and inexpensive. Most importantly, a mobile unit allows for conveyance between different farms, compared to the fruit being transported to a central location and it being exposed to further degradation during transit.

To further decrease the cost incurred, water could be sourced from boreholes, provided that the quality of the water is fit for human consumption. The only relatable cost may be from pumping, which can be addressed by implementing solar energy as a source of power.

Due to financial and logistical constraints, the IPCTU could not be operated in an orchard. However, the IPCTU was designed to fit in a 20-foot dry container. The dry container can be converted into a mobile packhouse to suit the user requirements. To create an environment conducive for workers, the following modifications are required:

1. Door × 2 large enough to allow the entry of the IPCTU and an entry and exit point for the fruit to avoid cross-contamination.
2. Door × 1 for the entry of personnel.
3. Windows × 3.
4. Adequate illumination with white light (Ladaniya, 2008).
5. Adequate ventilation (vent with exhaust fan).
6. Anti-condensation paint applied to interior.
7. Non-slip flooring.
8. Environmental control system.

Figure 6.3 illustrates the basic concept of a dry container required to house the IPCTU, which is envisioned for future research. However, during the current study the IPCTU

was housed in an agricultural tunnel constructed from polycarbonate sheets (40% transparency).

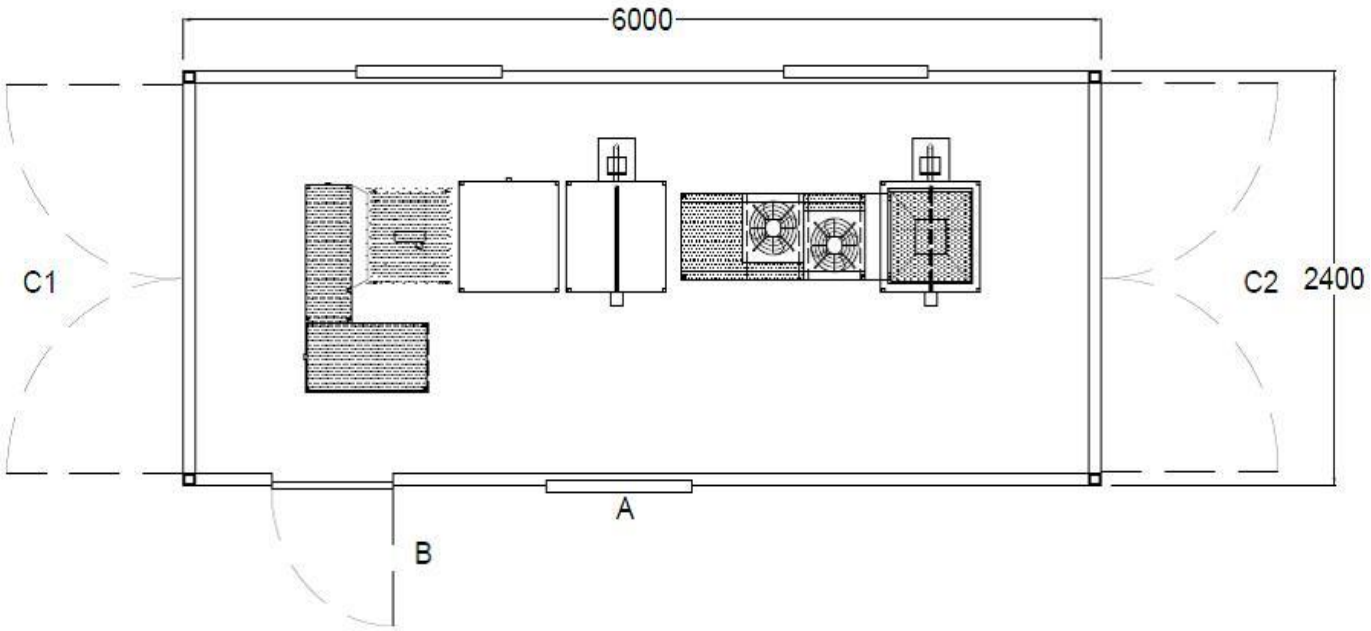

Figure 6.3 Schematic diagram of the IPCTU housed in a 20-foot dry container: A, window; B, door for personnel; C1, entry point of fruit; C2 exit point of fruit

6.8.3 Effect on fruit quality

6.8.3.1 Decay severity

Table 6.2 illustrates the decay severity of kumquat fruit due to *P. digitatum*. Treatments and the interactions of treatments with time were found to be significant ($P \leq 0.05$). No decay was observed for the first seven days for all treatments. However, between Day 7 and Day 14 mould formation was visible. The control treatment comprising of tap water (TW) + TW × 30s + No B13 (Treatment 36) resulted in the highest mould severity of 90% by Day 14. This was followed by 63.33% for Treatment 35 in which no anolyte water or hot water were used. The higher hot water temperature of 60°C proved to be slightly more beneficial in alleviating decay, compared to 53°C. However, Rodov *et al*. (2000) found that dipping kumquats in water heated to 53°C for 120s reduced decay. Similarly, Schirra *et al*. (2011) showed that dipping kumquat fruit in water heated to 50°C for 120s reduced decay. The differences in temperatures used in this study could be attributed to the shorter dipping times of only 20s and 30s. There was no major differences

observed between a hot water dipping time of 20s or 30s. However, 30s resulted in less decay as observed in Treatments 18 (2.33%), 20 (0%), 22 (15.67%) and 24 (6.67%).

The absence of the anolyte water promoted decay as was demonstrated by treatments in which tap water was used instead. This demonstrates the value of applying a disinfectant. The dipping time was also crucial. According to Table 6.2, 30s was slightly more beneficial compared to 60s as can be seen in Treatments 5 and 8, which resulted in decay of 5% and 48.33%, respectively. This was expected as disinfection is a crucial step in removing the micro-organisms existing on the fruit surface, which may lead to decay (Workneh *et al*., 2003). In comparison Treatments 1 and 4, in which a shorter dipping time was used, resulted in no decay. An acceptable disinfecting agent should have two main properties: (1) it should possess a sufficient level of antimicrobial activity; and (2) it should not interfere with the sensory quality of the fruit (Allende *et al*., 2008). Anolyte water was observed to adhere to both these properties. Gil *et al*. (2009) stated that a washing time exceeding 60 or 120 seconds had no improved capacity to reduce the bacterial count. The incorporation of yeast B13 as the final treatment reduced the decay relative to treatments that excluded B13. This was evident in Treatment 7 where B13 had been used (0%) and Treatment 8 where B13 was omitted (48.33%). Abraham *et al*. (2010) found that the yeast B13 (*C. fermentati*), was most effective as a preventative treatment.

Table 6.2 Changes in the decay severity (%) of kumquat fruit over a 14-day storage period subjected to different integrated pre-packaging treatments

Treatments	Storage Period (Days)		
	0	**7**	**14**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	0^{a}	0^{a}	0^{a}
Anolyte water × 30s + HWT × 20s × 53°C + No B13 (2)	0^{a}	0^{a}	0^{a}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (3)	0^{a}	0^{a}	0^{a}
Anolyte water × 30s + HWT × 30s × 53°C + No B13 (4)	0^{a}	0^{a}	0^{a}
Anolyte water × 60s + HWT × 20s × 53°C + B13 (5)	0^{a}	0^{a}	5^{bc}
Anolyte water × 60s + HWT × 20s × 53°C + No B13 (6)	0^{a}	0^{a}	0^{a}
Anolyte water × 60s + HWT × 30s × 53°C + B13 (7)	0^{a}	0^{a}	0^{a}
Anolyte water × 60s + HWT × 30s × 53°C + No B13 (8)	0^{a}	0^{a}	48.33^{j}
TW + HWT × 20s × 53°C + B13 (9)	0^{a}	0^{a}	25^{f}
TW + HWT × 20s × 53°C + No B13 (10)	0^{a}	0^{a}	1.67^{b}
TW + HWT × 30s × 53°C + B13 (11)	0^{a}	0^{a}	0^{a}
TW + HWT × 30s × 53°C + No B13 (12)	0^{a}	0^{a}	11.67^{cd}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (13)	0^{a}	0^{a}	0^{a}
Anolyte water × 30s + HWT × 20s × 60°C + No B13 (14)	0^{a}	0^{a}	0^{a}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (15)	0^{a}	0^{a}	0^{a}
Anolyte water × 30s + HWT × 30s × 60°C + No B13 (16)	0^{a}	0^{a}	0^{a}
Anolyte water × 60s + HWT × 20s × 60°C + B13 (17)	0^{a}	0^{a}	0^{a}
Anolyte water × 60s + HWT × 20s × 60°C + No B13 (18)	0^{a}	0^{a}	2.33^{b}
Anolyte water × 60s + HWT × 30s × 60°C + B13 (19)	0^{a}	0^{a}	0^{a}
Anolyte water × 60s + HWT × 30s × 60°C + No B13 (20)	0^{a}	0^{a}	0^{a}
TW + HWT × 20s × 60°C + B13 (21)	0^{a}	0^{a}	0^{a}
TW + HWT × 20s × 60°C + No B13 (22)	0^{a}	0^{a}	15.67^{e}
TW + HWT × 30s × 60°C + B13 (23)	0^{a}	0^{a}	0^{a}
TW + HWT × 30s × 60°C + No B13 (24)	0^{a}	0^{a}	6.67^{bc}
Anolyte water × 30s + TW × 20s + B13 (25)	0^{a}	0^{a}	15^{e}
Anolyte water × 30s + TW × 20s + No B13 (26)	0^{a}	0^{a}	6.33^{bc}
Anolyte water × 30s + TW × 30s + B13 (27)	0^{a}	0^{a}	15^{e}
Anolyte water × 30s + TW × 30s + No B13 (28)	0^{a}	0^{a}	28.33^{fg}
Anolyte water × 60s + TW × 20s + B13 (29)	0^{a}	0^{a}	10^{c}
Anolyte water × 60s + TW × 20s + No B13 (30)	0^{a}	0^{a}	0^{a}
Anolyte water × 60s + TW × 30s + B13 (31)	0^{a}	0^{a}	26.67^{f}
Anolyte water × 60s + TW × 30s + No B13 (32)	0^{a}	0^{a}	26.67^{f}
TW + TW × 20s + B13 (33)	0^{a}	0^{a}	30^{h}
TW + TW × 20s + No B13 (34)	0^{a}	0^{a}	63.33^{k}
TW + TW × 30s + B13 (35)	0^{a}	0^{a}	46.67^{i}
TW + TW × 30s + No B13 (36)	0^{a}	0^{a}	90^{l}
Significance			
Treatment (A)	*		
Day (B)	NS		
AB	*		
CV (%)	32.6		

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

Treatments which included anolyte water, hot water and the biocontrol agent resulted in less decay than fruit in which any one of these treatments were omitted. It is important to note that anolyte water, hot water and the biological control agent should not be seen in isolation from each other but rather this combination of treatments was found to complement each other and to be beneficial in terms of maintaining fruit quality. Based on these initial results the following were observed:

1. A dipping time in anolyte water of 30s was more beneficial than 60s in reducing decay.
2. A hot water temperature of 60°C and dipping time of 30s were most effective in reducing decay.
3. The presence of B13 resulted in zero decay.

A number of treatments resulted in zero decay as presented in Table 6.2 throughout the 14-day storage period. A more detailed analysis on the microbiological quality is required, which is presented in Chapter 7 – Section 7.6.6.

6.8.3.2 Physiological weight loss

Table 6.3 presents the physiological weight loss of kumquat fruit that were treated in the integrated system. The treatment, storage period and combination of these two factors were found to be highly significant ($P \leq 0.001$). The highest PWL was observed in control samples, corresponding to Treatment 36, of 65.60% on Day 14. Similar findings were observed for the decay severity (Table 6.2). Fruit treated with a dipping time of 30s for anolyte water generally produced a lower PWL than fruit dipped for 60s. The use of hot water resulted in a lower PWL, compared to using tap water. Hot water at 60°C was slightly less effective in reducing the PWL, than 53°C, which does not correspond to the findings for decay severity (Section 6.8.3.1). However, these findings agree with those of Rodov *et al*. (1995) and Schirra *et al*. (1995) in which fruit subjected to a hot water temperature of 53°C displayed lower weight loss. Rodov *et al*. (1995; 2000) and Ben-Yehoshua *et al*. (2005) suggested that heat treatments assist in redistributing the natural epicuticular wax, which seals microscopic cracks, preventing the escape of moisture, and therefore, promotes cell turgidity and firmer fruit. Hot water treatments were found to elicit plant host resistance against infection (Ben-Yehoshua *et al*., 2005).

Table 6.3 Changes in the physiological weight loss (%) of kumquat over a 14-day storage period subjected to different integrated pre-packaging treatments

Treatments	Storage Period (Days)		
	0	**7**	**14**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	0^{a}	6.10^{b}	35.24^{no}
Anolyte water × 30s + HWT × 20s × 53°C + No B13 (2)	0^{a}	15.57^{fg}	24.46^{jk}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (3)	0^{a}	16.26^{fgh}	18.25^{hi}
Anolyte water × 30s + HWT × 30s × 53°C + No B13 (4)	0^{a}	8.09^{bc}	22.06^{ij}
Anolyte water × 60s + HWT × 20s × 53°C + B13 (5)	0^{a}	9.19^{c}	40.74^{p}
Anolyte water × 60s + HWT × 20s × 53°C + No B13 (6)	0^{a}	2.39^{ab}	36.76^{no}
Anolyte water × 60s + HWT × 30s × 53°C + B13 (7)	0^{a}	13.63^{de}	27.52^{k}
Anolyte water × 60s + HWT × 30s × 53°C + No B13 (8)	0^{a}	9.65^{c}	24.77^{jk}
TW + HWT × 20s × 53°C + B13 (9)	0^{a}	15.75^{fg}	43.88^{pq}
TW + HWT × 20s × 53°C + No B13 (10)	0^{a}	15.39^{fg}	40.29^{p}
TW + HWT × 30s × 53°C + B13 (11)	0^{a}	17.27^{gh}	46.11^{q}
TW + HWT × 30s × 53°C + No B13 (12)	0^{a}	10.14^{cd}	34.79^{n}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (13)	0^{a}	10.61^{cd}	18.30^{hi}
Anolyte water × 30s + HWT × 20s × 60°C + No B13 (14)	0^{a}	19.76^{hij}	24.19^{jk}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (15)	0^{a}	18.07^{hi}	18.72^{hi}
Anolyte water × 30s + HWT × 30s × 60°C + No B13 (16)	0^{a}	29.94^{l}	36.78^{no}
Anolyte water × 60s + HWT × 20s × 60°C + B13 (17)	0^{a}	13.08^{de}	24.74^{jk}
Anolyte water × 60s + HWT × 20s × 60°C + No B13 (18)	0^{a}	16.31^{fgh}	31.50^{lm}
Anolyte water × 60s + HWT × 30s × 60°C + B13 (19)	0^{a}	15.83^{fg}	32.41^{lm}
Anolyte water × 60s + HWT × 30s × 60°C + No B13 (20)	0^{a}	19.74^{hij}	40.10^{p}
TW + HWT × 20s × 60°C + B13 (21)	0^{a}	13.98^{de}	38.64^{op}
TW + HWT × 20s × 60°C + No B13 (22)	0^{a}	31.33^{lm}	48.82^{r}
TW + HWT × 30s × 60°C + B13 (23)	0^{a}	7.87^{bc}	44.95^{pq}
TW + HWT × 30s × 60°C + No B13 (24)	0^{a}	8.61^{bc}	55.05^{s}
Anolyte water × 30s + TW × 20s + B13 (25)	0^{a}	14.10^{de}	40.68^{p}
Anolyte water × 30s + TW × 20s + No B13 (26)	0^{a}	18.61^{hi}	41.51^{p}
Anolyte water × 30s + TW × 30s + B13 (27)	0^{a}	12.17^{cde}	35.76^{no}
Anolyte water × 30s + TW × 30s + No B13 (28)	0^{a}	18.48^{hi}	45.42^{pq}
Anolyte water × 60s + TW × 20s + B13 (29)	0^{a}	14.88^{ef}	31.67^{lm}
Anolyte water × 60s + TW × 20s + No B13 (30)	0^{a}	14.41^{ef}	41.94^{p}
Anolyte water × 60s + TW × 30s + B13 (31)	0^{a}	20.11^{hij}	57.99^{st}
Anolyte water × 60s + TW × 30s + No B13 (32)	0^{a}	9.22^{c}	56.22^{st}
TW + TW × 20s + B13 (33)	0^{a}	11.25^{cde}	56.95^{st}
TW + TW × 20s + No B13 (34)	0^{a}	14.22^{de}	58.89^{stu}
TW + TW × 30s + B13 (35)	0^{a}	14.48^{ef}	57.41^{st}
TW + TW × 30s + No B13 (36)	0^{a}	12.60^{cde}	65.60^{v}
Significance			
Treatment (A)	**		
Day (B)	**		
AB	**		
CV (%)	39.2		

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

The loss in weight is often accompanied by a decrease in firmness (Porat *et al*., 1999). Treatment 3 (Anolyte water × 30s + HWT × 30s × 53°C + B13), Treatment 13 (Anolyte water × 30s + HWT × 20s × 60°C + B13) and Treatment 15 (Anolyte water × 30s + HWT × 30s × 60°C + B13) produced the lowest PWL values of 18.25%, 18.30% and 18.72%, respectively. The addition of B13 as a treatment, produced fruit with lower PWL values than if B13 was omitted.

6.8.3.3 Peel firmness

The change in the peel firmness of kumquat fruit as a result of the different pre-packaging treatments is presented in Table 6.4. All factors and interactions were found to be highly significant ($P \leq 0.001$). Treatments 33, 34 and 36 gave rise to the least firm fruit of 1.78 N, 1.82 N and 0.94 N by Day 14, respectively, corresponding to the higher decay severity values of 30%, 63.33% and 90%, respectively (Table 6.2). An observation that was consistent for the decay severity, PWL and peel firmness is that the control treatment (Treatment 36) displayed the poorest quality. A decrease in the peel firmness from Day 0 to Day 7 can be observed in Table 6.4. However, in certain treatments, such as Treatments 4, 6, 7 and 13, an increase in the firmness was noted between Day 7 and Day 14. This can be attributed to an increase in the moisture loss, which results in the citrus fruit peel becoming tough and leathery (Ladaniya, 2008). This causes a higher puncture resistance and could account for an increase in the peel firmness by Day 14. In other instances, there was a notable increase in the firmness from Day 0 to Day 7 followed by a decrease (Treatment 8). This could be attributed to a loss in moisture, resulting in a tough leathery skin by Day 7 before the fruit succumbed to decay, resulting in softening of the peel. Li *et al*. (2010) also reported an increase in fruit firmness after harvest. This can be attributed to physical damage, and storage at low temperatures or at temperatures up to 20°C, resulting in cell wall secondary lignification. The highest fruit firmness was triggered by Treatment 17 of 16.78 N on Day 14. The drastic increase in firmness could have been due to the hot water treatment, which may have led to cell wall secondary lignification due to the high treatment temperature of 60°C. Treatment 1 (Anolyte water × 30s + HWT × 20s × 53°C + B13) and Treatment 18 (Anolyte water × 60s + HWT × 20s × 60°C + No B13) resulted in the least variation in the peel firmness from Day 0 to Day 14.

Table 6.4 Changes in the peel firmness (N) of kumquat fruit over a 14-day storage period subjected to different integrated pre-packaging treatments

Treatments	Storage Period (Days)		
	0	**7**	**14**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	12.21^{gh}	12.72^{gh}	11.66^{fg}
Anolyte water × 30s + HWT × 20s × 53°C + No B13 (2)	11.99^{fg}	13.43^{ghi}	10.13^{efg}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (3)	12.8^{gh}	10.35^{efg}	12.61^{gh}
Anolyte water × 30s + HWT × 30s × 53°C + No B13 (4)	11.66^{fg}	4.55^{cde}	13.16^{ghi}
Anolyte water × 60s + HWT × 20s × 53°C + B13 (5)	13.43^{ghi}	12.73^{gh}	14.5^{hij}
Anolyte water × 60s + HWT × 20s × 53°C + No B13 (6)	10.35^{efg}	4.12^{cde}	13.4^{ghi}
Anolyte water × 60s + HWT × 30s × 53°C + B13 (7)	11.66^{fg}	7.35^{de}	10.74^{efg}
Anolyte water × 60s + HWT × 30s × 53°C + No B13 (8)	13.43^{ghi}	18.7^{k}	13.75^{hi}
TW + HWT × 20s × 53°C + B13 (9)	10.35^{efg}	3.76^{cd}	12.34^{gh}
TW + HWT × 20s × 53°C + No B13 (10)	11.66^{fg}	12.24^{gh}	11.21^{fg}
TW + HWT × 30s × 53°C + B13 (11)	13.43^{ghi}	10.15^{efg}	9.89^{efg}
TW + HWT × 30s × 53°C + No B13 (12)	10.35^{efg}	13.45^{ghi}	15.01^{hij}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (13)	11.66^{fg}	9.31^{ef}	15.43^{ij}
Anolyte water × 30s + HWT × 20s × 60°C + No B13 (14)	13.43^{ghi}	10.69^{efg}	10.38^{efg}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (15)	10.35^{efg}	7.22^{de}	12.51^{gh}
Anolyte water × 30s + HWT × 30s × 60°C + No B13 (16)	11.66^{fg}	9.39^{ef}	11.47^{fg}
Anolyte water × 60s + HWT × 20s × 60°C + B13 (17)	13.43^{ghi}	13.8^{hi}	16.78^{j}
Anolyte water × 60s + HWT × 20s × 60°C + No B13 (18)	10.35^{efg}	10.55^{efg}	11.46^{fg}
Anolyte water × 60s + HWT × 30s × 60°C + B13 (19)	11.66^{fg}	13.98^{hi}	14.11^{hij}
Anolyte water × 60s + HWT × 30s × 60°C + No B13 (20)	13.43^{ghi}	13.97^{hi}	14.57^{hij}
TW + HWT × 20s × 60°C + B13 (21)	10.35^{efg}	6.46^{de}	11.09^{fg}
TW + HWT × 20s × 60°C + No B13 (22)	11.66^{fg}	11.63^{fg}	11.24^{fg}
TW + HWT × 30s × 60°C + B13 (23)	13.43^{ghi}	13.17^{ghi}	11.97^{fg}
TW + HWT × 30s × 60°C + No B13 (24)	10.35^{efg}	12.72^{gh}	13.43^{ghi}
Anolyte water × 30s + TW × 20s + B13 (25)	11.66^{fg}	9.74^{ef}	14.92^{hij}
Anolyte water × 30s + TW × 20s + No B13 (26)	13.43^{ghi}	13.76^{ghi}	13.71^{hi}
Anolyte water × 30s + TW × 30s + B13 (27)	10.35^{efg}	6.82^{de}	13.34^{ghi}
Anolyte water × 30s + TW × 30s + No B13 (28)	11.66^{fg}	3.48^{cd}	12.31^{gh}
Anolyte water × 60s + TW × 20s + B13 (29)	13.43^{ghi}	5.17^{cde}	13.77^{hi}
Anolyte water × 60s + TW × 20s + No B13 (30)	10.35^{efg}	6.15^{de}	12.22^{gh}
Anolyte water × 60s + TW × 30s + B13 (31)	11.66^{fg}	11.25^{fg}	12.54^{gh}
Anolyte water × 60s + TW × 30s + No B13 (32)	13.43^{ghi}	2.72^{bc}	10.40^{efg}
TW + TW × 20s + B13 (33)	10.35^{efg}	12.54^{gh}	1.78^{abc}
TW + TW × 20s + No B13 (34)	11.66^{fg}	0.83^{a}	1.82^{abc}
TW + TW × 30s + B13 (35)	13.43^{ghi}	2.27^{bc}	4.52^{cde}
TW + TW × 30s + No B13 (36)	10.35^{efg}	1.82^{abc}	0.94^{a}
Significance			
Treatment (A)	**		
Day (B)	**		
AB	**		
CV (%)	13.4		

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

6.9 Conclusion

The aim of this study was to design an IPCTU capable of treating kumquat fruit immediately after harvest at the orchard employing integrated environmentally friendly treatments of anolyte water, hot water and a yeast bicontrol agent (B13). The choice of these specific treatments were made based on a previous laboratory-based experiment (Chapter 5). The IPCTU was constructed from 0.9 mm thick 304 stainless steel (food grade) with six distinct treatments zones: (1) primary rinsing, (2) secondary rinsing (required if fruit has excessive amount of field dirt), (3) anolyte water – disinfection treatment, (4) hot water – curative treatment, (5) surface moisture removal, and (6) B13 biocontrol agent – preventative treatment (Kassim *et al*., 2016). The main treatments of anolyte water, hot water and B13 work in a complementary manner with different modes of action to reduce decay and improve fruit quality. The disinfectant treatment is applied to the surface debris picked up on fruit in the field (Workneh *et al*., 2003, Kassim *et al*., 2016). The hot water treatments seals surface cracks and fissures so as to prevent the loss of moisture and the entry point for pathogens (Ben-Yehoshua *et al*., 2005). In addition, the hot water treatment has the ability to induce the plant host resistance, thereby being a curative treatment. The biocontrol agent is used as a preventative treatment to prevent further infection from pathogens (Abraham *et al*., 2010).

The power consumptions for Tank D (hot water) and Tank F (B13) was 4.13 kW and 2.08 kW, respectively. The energy required by Tank D was twice as much as Tank F due to the higher treatment temperatures of up to 60°C. Glasswool insulation was installed around Tank D to prevent the loss of energy to the surroundings. The insulation resulted in an energy saving of 243.50 J, compared to if no insulation was used. More energy was lost from the system due to convection than radiation. The thermal efficiency of Tank D was 72% and Tank F was found to be 87% efficient. The total cost of the system was attributed to fixed costs (construction labour and materials) and variable costs (electricity, water, labour for operation and consumables). This resulted in a payback period of 0.91 years. The carbon ratio was determined as 0.46 kg $CO_2.day^{-1}$. The tank operated outdoors under non-laboratory conditions. Due to financial and logistical constraints, the IPCTU could not be operated in a kumquat orchard. However, the IPCTU operated effectively during the experiment.

A total of 36 combination treatments were tested to determine the treatment/s, which was most beneficial in terms of the decay, PWL and peel firmness of kumquat fruit. Treatment 3 (Anolyte water × 30s + HWT × 30s × 53°C + B13), Treatment 13 (Anolyte water × 30s + HWT × 20s × 60°C + B13) and Treatment 15 (Anolyte water × 30s + HWT × 30s × 60°C + B13) produced the lowest PWL values of 18.25%, 18.30% and 18.72%, respectively. Treatment 1 (Anolyte water × 30s + HWT × 20s × 53°C + B13) and Treatment 18 (Anolyte water × 60s + HWT × 20s × 60°C + No B13) resulted in the least variation in the peel firmness from Day 0 to Day 14. Based on the results the following treatments were screened further to determine the most effective treatment of kumquat fruit using the IPCTU:

1. Treatment 1: Anolyte water × 30s + HWT × 20s × 53°C + B13
2. Treatment 2: Anolyte water × 30s + HWT × 30s × 53°C + B13
3. Treatment 3: Anolyte water × 30s + HWT × 20s × 60°C + B13
4. Treatment 4: Anolyte water × 30s + HWT × 30s × 60°C + B13

These treatments include a combination of the three main treatments of (1) anolyte water, (2) hot water and (3) B13 at the exposure times and temperatures that were common, which resulted in the most beneficial effect on the decay severity, PWL and peel firmness of the kumquat fruit. A more comprehensive evaluation of the physical, chemical, microbiological and subjective quality of kumquat fruit subjected to these treatment conditions is provided in Chapter 7.

6.10 References

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7. TREATMENT COMPARISON OF KUMQUAT FRUIT USING THE POSTHARVEST CITRUS TREATMENT UNIT FOR TRANSPORT CONDITIONS SIMULATION

7.1 Abstract

Environmentally friendly treatments are required to alleviate decay caused by the *Penicillium* spp. pathogen in kumquat fruit. This is of particular importance due to the manner in which the fruit is consumed, which includes the peel. The use of an integrated postharvest citrus treatment unit (IPCTU) was been used to treat kumquat fruit, which included three main treatments of (1) anolyte water, (2) hot water and (3) B13 biocontrol agent. Previous studies have demonstrated the beneficial effect of combining these three treatments in reducing decay caused by *P. digitatum* and *P. italicum*. Therefore, this study aimed to determine the optimal treatment regime capable of reducing and delaying the onset of decay caused by *P. digitatum* and *P. italicum* and maintaining the kumquat fruit quality. Furthermore, this study analysed the effect of pre-packaging treatments on the physical (physiological weight loss (PWL), peel colour, peel firmness); chemical (peel moisture content (MC), total soluble solids (TSS)); microbiological (total aerobic plate count (APC), total coliform count (CC), total fungal count (FC)) and the subjective quality of kumquat fruit, which has not been documented thus far. 10 uL *P. digitatum* and *P. italicum* were each inoculated into 7.5 kg of kumquat fruit at 1×10^4 conidia.mL^{-1}, which was then treated using the IPCTU. Treatments, storage period and the interaction of these factors had a significant influence on each of the parameters at either $P \leq 0.05$ or $P \leq 0.001$. Control samples (no treatment) displayed the poorest quality. *P. digitatum*-inoculated fruit treated with anolyte water × 30s + HWT × 20s × 53°C + B13 (Treatment 1) exhibited the best quality. Treatment 2 (anolyte water × 30s + HWT × 30s × 53°C + B13) resulted in fruit possessing the best quality for *P. italicum*-inoculated fruit. *P. digitatum*-inoculated fruit exhibited a low PWL (37.87%), firm fruit (6.20 N), high MC (58.3%), low TSS (11.2 °Brix) by Day 21 and a low CC (5.6 log CFU.g^{-1}) immediately after treatment when treated with Treatment 1. Treatment 2 resulted in low PWL (27.72%), reduced colour change (70.92°), low MC (62.2%), low TSS (10.7 °Brix) on Day 21 and a low CC (5.5 log CFU.g^{-1}) and FC (5.7 log CFU.g^{-1}) immediately after treatment for *P. italicum*-inoculated fruit. Visible mould formation was observed only on

control samples on Day 14. Treatment 2 only slightly outperformed Treatment 1 with regard to the overall quality of the fruit for *P. italicum*-inoculated fruit. However, Treatment 1 outperformed Treatment 2 to a higher degree for *P. digitatum*-inoculated fruit. It is, therefore, recommended that Treatment 1 be applied using the postharvest citrus treatment unit on kumquat fruit as *P. digitatum* is the more prevalent pathogen infecting citrus fruit.

7.2 Introduction

Kumquat fruit remain a relatively small contributor to the citrus crop in South Africa with only 160 000 cartons being passed for export during the 2014/2015 season (Department of Agriculture, Forestry and Fisheries, 2015). However, the South African production and export of kumquat fruit has increased from the 2013/2014 to 2014/2015 season. The advantage of exporting kumquat fruit from South Africa is due to it being a niche fruit, as it is sold to the European Union from May to October each year, unlike other countries (Beghin, 2014). Other citrus fruit such as oranges, lemons, grapefruit and mandarins take up a large portion of the commercial farming sector. This creates an opportunity for small-scale farmers to get involved in the kumquat market, possibly contributing to investments in terms of human resources and foreign exchange earnings.

Kumquat fruit are associated with high transpiration rates and are susceptible to decay due to *Penicillium digitatum* and *P. italicum* (Li *et al*., 2008; Schirra *et al*., 2011). In South Africa, once harvested, the kumquat fruit are transported to packhouses in unrefrigerated trucks, usually with other fruit. This is undesirable because fruit are exposed to increased temperatures during transport and the commodity transported alongside the kumquat may not necessarily be compatible, which could hasten the ripening process (TransFresh Corporation, 1999). At the packhouse the kumquats are typically disinfected with chlorinated water (1% chlorine solution) subsequent to rinsing before being air dried. However, Kassim *et al*. (2016) found that chlorinated water treatment may result in excessive weight loss in kumquat fruit. The United States Food and Drug Administration (2014) permits the use of chlorine dioxide as an antimicrobial agent for treating fruits and vegetables that are not raw agricultural commodities to which the level of residue shall not exceed 3 $mg.kg^{-1}$. Previously, kumquat treatments were selected based on the compatibility of the treatment with the available equipment due to

the relatively small quantity of fruit (Hall, 1986). However, consumers are becoming more aware of the treatments being applied to food that they consume and regulations are more stringent with regard to the treatments implemented in the food processing industry. This has forced researchers to seek and develop more environmentally friendly treatments of horticultural commodities. There still exists a dearth of research focusing on the pre-packaging treatment and quality of kumquat fruit. A limited number of studies have been performed to find alternative treatments to replace the use of synthetic chemicals and fungicides to reduce postharvest decay in kumquats, compared to other citrus (Ben-Yehoshua *et al*., 2005; Li *et al*., 2008; Schirra *et al*., 2011; Kassim *et al*., 2016).

Hot water treatments in the temperature range of 50°C to 56°C for an exposure period of 30 seconds to 120 seconds have been found favourable in maintaining the quality of kumquat fruit and reducing the severity of *P. digitatum* and *P. italicum* infections (Rodov *et al*., 1992; Rodov *et al*., 1995; Ben-Yehoshua *et al*., 2000; Schirra *et al*., 2008). Compared to other heat treatments such as curing, hot water treatments pose less challenges, are less expensive and are more feasible at an industrial scale (Garcia *et al*., 2016). Anolyte water has been used on horticultural commodities with success as a disinfectant as in the case of carrots, peaches, grapes and kumquats (Workneh *et al*., 2003; Guentzel *et al*., 2010; Kassim *et al*., 2016). Biocontrol agents has been recommended as an environmentally friendly method of treating citrus (Abraham *et al*., 2010; Kassim *et al*., 2016).

This study was based on previous research conducted by Kassim *et al*. (2016) documented in Chapter 5 and combined with the findings of Chapter 6. Chapter 5 revealed that integrating the pre-packaging treatments of anolyte water, hot water and a biocontrol agent were effective in reducing decay and maintaining kumquat fruit quality on a laboratory scale, compared to individual treatments. Chapter 6 demonstrated the use of an IPCTU to apply these treatments to kumquat fruit at a pilot scale. This study was designed to refine the findings of Chapters 5 and 6 to identify the most suitable treatment to maintain the best quality of kumquat fruit using the IPCTU. A more comprehensive analytical approach was adopted in determining the fruit quality encompassing the physical, chemical, microbiological and subjective quality analyses of the kumquat fruit. In addition, this Chapter simulates transport conditions by subjecting the fruit to a temperature of 4.5°C and 55.2% relative humidity for a 21-day period. It was envisaged

that the outcome of this chapter would assist small-scale farmers in the processing of kumquat fruit using the IPCTU together with the optimal treatment regime.

The specific objectives formulated for this study were to determine the:

1. Effect of different pre-packaging treatments on the physical, chemical, microbiological and subjective quality of kumquat fruit.
2. Optimal treatment for kumquat fruit during the commercially recommended temperature of at 4.5°C.

7.3 Materials and Methods

7.3.1 Sample fruit production

The kumquat fruit were obtained from Rooister Boerdery in the Letsitele region, just outside Tzaneen, Limpopo Province, South Africa, as mentioned in Chapters 4, 5 and 6. A total of 13 kg were used for this study. Fruit samples were visually inspected for damage or irregularity in shape, size and colour. Fruit of a similar size, colour, shape and free of imperfections were selected for further investigation (Hong *et al*., 2007; Schirra *et al*., 2011).

7.3.2 Pre-packaging treatments

The four treatments, which performed optimally in Chapter 6 were selected for further analysis using the IPCTU. In addition, a wash using tap water and no treatment were used. The treatments used in this study are as follows:

1. Treatment 1: Anolyte water × 30s + HWT × 20s × 53°C + B13
2. Treatment 2: Anolyte water × 30s + HWT × 30s × 53°C + B13
3. Treatment 3: Anolyte water × 30s + HWT × 20s × 60°C + B13
4. Treatment 4: Anolyte water × 30s + HWT × 30s × 60°C + B13
5. Treatment 5: Tap water × 30s
6. Treatment 6: No treatment.

7.3.3 Experimental design

The experiments were full factorial. The fruit were sorted into 36 batches of 6 fruit each and labelled at the base of the fruit using a white marker. Half the number of samples (18 batches) were inoculated with *P. digitatum* and the remaining half were inoculated with *P. italicum* prior to treatment application as described further in Section 7.3.6.

7.3.4 Isolation of *Penicillium digitatum* and *Penicillium italicum* from infected fruit

The method explained in Chapter 5, Section 5.3.4 was followed.

7.3.5 Sample preparation

Untreated kumquat fruit were inspected based on uniformity of size, colour and damage (Hong *et al*., 2007). Fruit that showed signs of damage or deformity were discarded. After treatment, all samples were stored within a cold room at the recommended storage temperature of 4.5°C and 55.2% relative humidity for a period of 21 days, to simulate commercial transport conditions. The standard shipping duration for exporting kumquats from South Africa to the European Union is 14 days (Steyn, 2016). However an additional 7 days were added to determine the effect of the different treatments, should there be a delay in shipping. Fruit were sampled on Days 0 (before and after treatments), 7, 14, and 21 for the physical, chemical, microbiological and subjective quality. Due to a lower storage temperature adopted to simulate kumquat transport conditions, *P. italicum* was used in addition to *P. digitatum* as the inoculants (Brown, 1994).

7.3.6 Inoculation of kumquat using *Penicillium digitatu*m and *Penicillium italicum*

P. digitatum and *P. italicum* conidia that had been prepared as explained in Section 7.3.4 were used. The method explained in Chapter 5, Section 5.3.6 for the inoculation of *P. digitatum* was followed.

7.4 Data Collection and Analysis

The effect of the treatments on the kumquat fruit were evaluated for changes in the physical, chemical, microbiological and subjective quality of the fruit. The physical quality parameters that were investigated included the physiological weight loss, peel colour (hue angle) and peel firmness. The chemical quality parameters that were investigated included the peel moisture content and total soluble solids. The microbiological analysis was based on an estimate of the total (1) aerobic microorganism population, (2) coliform population, and (3) fungal population. Lastly, the subjective quality analysis included the visual inspection of each fruit sample.

7.4.1 Physiological weight loss

Kumquat fruit were individually weighed using a Mettler PJ 300 scale (Mettler-Toledo, Barcelona, Spain) at the start of the experiment and at the specified sampling intervals. The differential weight loss was calculated for each sample per interval and converted to a percentage of the original fresh weight of the fruit (wet basis) (Singh and Reddy, 2006; Hong *et al*., 2007).

7.4.2 Peel colour

The hue angle of the kumquat peel was measured using a Konica Minolta CR-400 colorimeter (Konica Minolta Inc., Osaka, Japan) (Li *et al*., 2008). The instrument was calibrated using the white calibration tile and set with a C illuminant. An average of three readings around the equatorial region per fruit was measured.

7.4.3 Peel firmness

An Instron Universal Testing Machine (Model 3345) (Advanced Laboratory Solutions, Baar, Switzerland) was used in conjunction with the Instron Bluehill 2 Version 2.25 software to determine the firmness of the kumquat peel by means of puncturing the fruit surface. Individual unpeeled kumquat fruit were placed horizontally on the curved platform (stem axis parallel to plate). A probe of 1.5 mm diameter was used to perform two punctures per fruit sample on opposite sides of the equatorial region. The cross head

speed was set at 200 mm.min^{-1} to travel to a depth of 12 mm. The maximum force required to puncture the fruit was taken as the exterior fruit firmness (Valero *et al*., 1998).

7.4.4 Peel moisture content

Each fruit was cut in half with the pulp removed from one half. 2 g of the peel was placed on a piece of aluminium foil. The weight of the foil and peel were measured using a Mettler PJ 300 scale (Mettler-Toledo, Barcelona, Spain). The samples were then placed in a hot air oven at 105°C for 24 hours (Jaliliantabar *et al*., 2013). After drying for 24 hours, the samples were reweighed. The peel moisture content was calculated on a wet basis (Singh and Reddy, 2006).

7.4.5 Total soluble solids

The total soluble solids expressed as °Brix was determined by extracting juice from the pulp of each fruit and placing it on the prism of the Atago digital hand-held "pocket" refractometer (±0.2 % accuracy) (ATAGO USA Inc., Washington, USA) (Valero *et al*., 1998; Schirra *et al*., 2011). The prism was cleaned with 99.9% ethanol and then distilled water, using a soft cloth between samples.

7.4.6 Microbiological

Three fruit were placed in a sterile plastic bag with 30 mL 0.1% sterile peptone water (per litre of distilled water; 8.5 g of NaCl and 1 g of peptone, pH 7). The fruit were massaged for 2 minutes to loosen and dislodge any surface microbes. The slurries were aseptically, serially diluted in 9 mL 0.1% sterile peptone water. A further serial decimal dilution was performed up to 10^{-2}, using methods described by Sibomana *et al*. (2016). To determine the population of total aerobic microorganisms, triplicate samples were plated on plate count agar (pH 7.0±0.2, Oxoid, Basingstoke, England) and incubated at 30ºC for 2 days. For estimating coliform populations, triplicate samples were plated on violet red bile agar (with MUG, Oxoid, Basingstoke, England) and incubated at 37ºC for 1 day. To determine fungi and yeasts, triplicate samples were plated on rose Bengal (with chloramphenicol) agar (Oxoid, Basingstoke, England) and incubated at room temperature for 3 to 5 days. The spread plate method was used to transfer 0.1 uL of the 10^{-2} serial dilution onto each

respective plate. Colonies were counted after the required incubation period for each of the three microorganisms and the results expressed as the mean number of colony-forming units (CFU) per gram (El-Ghaouth *et al*., 2000).

7.4.7 Subjective quality analysis

The marketability was determined by visual observation according to the criteria used by Adekalu and Agboola (2015) on a 6-point hedonic scale of 1-6, where 1 = very poor, 2 = poor, 3 = fair, 4 = good, 5 = very good and 6 = excellent. The physical characteristics considered included any physical damage that may have appeared during storage, peel discolouration, peel shininess and smoothness, watery texture and the onset of decay or appearance of mould.

7.4.8 Overall ranking of treatment

To create a better perspective on the effectiveness of each of the six treatments, a number from 1 to 6 was allocated to each treatment at the end of the storage period (Day 21). This number was allocated to a treatment based on how effectively the treatment maintained or improved each of the quality parameters, compared to other treatments. A number '1' presented the best performing treatment, while '6' represented the worst performing treatment.

7.5 Statistical Data Analysis

The statistical analysis was performed using the GenStat software, 17th Edition. The differences between treatments were determined by an analysis of variance (ANOVA) and the means were separated using the Duncan's Multiple Range Test, with a significance level of 0.05 used to establish differences between mean values.

7.6 Results and Discussion

7.6.1 Physiological weight loss

Table 7.1 presents the effect of different treatments on the physiological weight loss (PWL) of *P. digitatum*-inoculated kumquat fruit. The treatment, storage period and the interaction of both these factors were found to have a highly significant (P≤0.001) influence on the PWL. Fruit with no treatment displayed the highest PWL of 66.83% by Day 21. Fruit subjected to a temperature of 60°C displayed a higher PWL, compared to treatments at 53°C.

Table 7.1 Changes in the physiological weight loss (%) of *Penicillium digitatum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	0^{a}	19.93^{bc}	22.39^{bcd}	27.68^{de}	37.87^{fg}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	0^{a}	23.39^{bcd}	27.02^{de}	32.68^{f}	41.93^{gh}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	0^{a}	24.38^{cd}	24.38^{cd}	29.52^{ef}	62.83^{ij}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	0^{a}	22.39^{bcd}	28.48^{def}	32.20^{f}	62.71^{ij}
TW × 30s (5)	0^{a}	16.93^{b}	31.14^{f}	31.69^{f}	58.81^{hi}
No treatment (6)	0^{a}	18.56^{bc}	23.61^{cd}	29.62^{ef}	66.83^{j}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	**				
CV (%)	51.8				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

Table 7.2 presents the effect of different pre-packaging treatments on the PWL of *P. italicum*-inoculated kumquat fruit. The treatment, storage period and the interaction of both these factors were found to have a highly significant (P≤0.001) influence on the PWL. The highest cumulative PWL was observed for samples with no treatment amounting to 48.40%, which was considerably higher than other treatments by Day 21.

Fruit treated at 53°C for 30 seconds displayed a slightly lower PWL 27.72%, compared to fruit treated at the same temperature for 20 seconds of 36.08%. Fruit treated at 60°C for 30 seconds displayed a lower PWL, compared to those treated for 20 seconds until Day 14. Thereafter no significant difference in the PWL was identified on Day 21. The results obtained are comparable to those encountered for *P. digitatum*-inoculated fruit.

Table 7.2 Changes in the physiological weight loss (%) of *Penicillium italicum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	0^{a}	15.45^{bc}	22.78^{cde}	33.25^{ef}	36.08^{fg}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	0^{a}	14.00^{ab}	21.06^{cde}	24.49^{de}	27.72^{def}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	0^{a}	18.02^{cd}	32.59^{ef}	33.06^{ef}	36.08^{fg}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	0^{a}	13.66^{ab}	14.34^{abc}	28.16^{def}	37.08^{fg}
TW × 30s (5)	0^{a}	18.02^{cd}	22.58^{cde}	28.66^{ab}	39.52^{g}
No treatment (6)	0^{a}	27.59^{def}	29.28^{def}	31.46^{ef}	48.40^{h}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	**				
CV (%)	36.4				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

The PWL drastically increased from Day 0B to Day 0A for both the *P. digitatum*- and *P. italicum*-inoculated fruit. Increased levels of PWL can be attributed to the higher temperatures triggering an immediate rise in the transpiration rate of the fruit, which is mainly affected by the surrounding temperature (Hong *et al*., 2007). Too low or too high water treatment temperatures can be detrimental to fruit by resulting in a high PWL. This concept was demonstrated by Garcia *et al*. (2016) and Palma *et al*. (2013). Garcia *et al*. (2016) found that oranges subjected to water temperatures of 40°C resulted in a higher weight loss, compared to 53°C. Palma *et al*. (2013) demonstrated that a temperature of 59°C for 'Tarocco' oranges was too close to the threshold temperature, which could result is physiological damage. Too high temperature may also reduce the efficacy of other

treatments that are used in combination with the hot water. Too high a temperature can result in excessive melting and removal of the natural epicuticular wax, negatively affecting fruit quality (Ben-Yehoshua, 2005). More importantly is the time and temperature combination at which the fruit is exposed to the hot water. The conventional heating process consists of convective heat transfer from the heating medium to the fruit surface, thereafter conductive heat transfer from the surface to the fruit center occurs (Wang *et al*., 2001).

These findings appear to be in accordance to those found by Schirra *et al*. (1995), Ben-Yehoshua *et al*. (2000), Rodov *et al.* (2000), Palma *et al*. (2013) and Garcia *et al*. (2016) where the optimal treatment temperatures was found to be in the range of 53°C with regard to the PWL of kumquat fruit. In addition, this study revealed that the shorter treatment times were beneficial, compared to longer treatments times as studies by Ben-Yehoshua *et al*. (2000) and Rodov *et al.* (2000) have shown, which is well-suited for industrial applications.

Based on these results, it can be deduced that *P. digitatum*-inoculated kumquat fruit treated with anolyte water × 30s + 53; 20s HWT × 30s × 53°C + B13 and *P. italicum*-inoculated kumquat fruit treated with anolyte water × 30s + 53; 30s HWT × 30S × 53°C + B13 led to the least loss in weight at the end of the 21-day storage period.

7.6.2 Peel colour

The change in the hue angle of *P. digitatum*-inoculated kumquat fruit subjected to different pre-packaging treatments are presented in Table 7.3. The treatment, storage period and the interaction of both these factors were found to have a level of significance of $P \leq 0.05$. Control samples displayed the lowest hue angle by Day 21 of 66.58°. Treatments 5 and 6 resulted in the greatest reduction of the hue angle from Day 0B to Day 21 of 7.5% and 9.2%, respectively. Treatment 4 produced fruit with the least reduction in the hue angle of only 1.4% from Day 0B to Day 21, followed by Treatment 3 with a reduction of 3.1%. This demonstrates that a treatment temperature of 60°C decreased the rate of change in the hue angle of kumquat fruit.

Table 7.3 Changes in the hue angle (°) of *Penicillium digitatum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	73.17^{cd}	71.45^{abc}	70.74^{ab}	70.05^{ab}	70.64^{ab}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	73.17^{cd}	72.92^{bcd}	72.31^{bc}	71.55^{bc}	67.98^{ab}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	73.17^{cd}	73.84^{cd}	72.7^{bcd}	72.92^{bcd}	70.91^{abc}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	73.17^{cd}	72.14^{bc}	74.00^{cd}	72.19^{bc}	72.08^{bc}
TW × 30s (5)	73.17^{cd}	71.58^{bc}	72.93^{bcd}	71.16^{abc}	67.71^{ab}
No treatment (6)	73.17^{cd}	71.52^{bc}	67.63^{ab}	67.64^{ab}	66.58^{a}
Significance					
Treatment (A)	*				
Storage Period (B)	*				
AB	*				
CV (%)	3.5				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

Table 7.4 presents the changes in the kumquat peel hue angle for fruit inoculated with *P. italicum*. The treatment, storage period and the interaction of these factors were found to be significant at P≤0.05. A general decrease in the hue angle was observed for all treatments. The decrease in the hue angle was most apparent at the start of the storage period, however, as time progressed, the rate at which the hue angle decreased lessened. Treatments 5 and 6 demonstrated the greatest reduction in the hue angle from Day 0B to Day 21 equating to an 8.0% (73.20° to 67.41°) and 6.4% (73.20° to 68.51°) reduction, respectively. Treatments 2, 3 and 4 displayed similar changes throughout the storage period with a 3% reduction from Day 0B to Day 21, representative of the least reduction in the hue angle. Treatment 1 exhibited a slightly higher reduction of 4% from 73.20° on Day 0B to 69.94° on Day 21.

Table 7.4 Changes in the hue angle (°) of *Penicillium italicum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	73.20^{cd}	71.21^{abc}	69.97^{ab}	70.95^{abc}	69.94^{ab}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	73.20^{cd}	72.50^{bc}	72.93^{cd}	69.37^{ab}	70.92^{abc}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	73.20^{cd}	73.00^{cd}	72.80^{bc}	72.50^{bc}	71.10^{abc}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	73.20^{cd}	71.45^{abc}	71.39^{abc}	71.31^{abc}	71.05^{abc}
TW × 30s (5)	73.20^{cd}	73.00^{cd}	69.73^{ab}	71.19^{abc}	67.41^{a}
No treatment (6)	73.20^{cd}	72.90^{bc}	70.36^{abc}	66.87^{a}	68.51^{ab}
Significance					
Treatment (A)	*				
Storage Period (B)	*				
AB	*				
CV (%)	3.5				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

Rodov *et al*. (2000) observed a considerable delay in yellowing of 'Oroblanco' fruit when treated at 60°C. According to Karcher and Richardson (2003) a hue angle of 0° is representative of red, 60° is representative of yellow and 120° is representative of green. Therefore, a reduction in the hue angle within the range shown in Tables 7.3 and 7.4 can be described as a colour change from yellow-lime to orange-yellow, indicative of ripening. This change is due to the natural replacement of chloroplasts with chromoplasts in the fruit epicarp (Iglesias *et al*., 2001). The rapid change in the hue angle at the start of the storage period can be attributed to the increase in the rate of respiration due to the exposure of the fruit to increased temperatures. However, during storage the change in these characteristics became less apparent (Hong *et al*., 2007).

Based on the results, it can be deduced that *P. digitatum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 20s × 60°C + B13 and *P. italicum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 30s × 53°C + B13 or anolyte water × 30s + HWT × 20s × 60°C + B13 or anolyte water × 30s + HWT × 20s × 60°C + B13 developed

the least change in the hue angle of the peel at the end of the 21-day storage period.

7.6.3 Peel firmness

Table 7.5 presents the variation in the peel firmness of *P. digitatum*-inoculated kumquat fruit. The treatment, storage period and the interaction of both the treatment and the storage period were found to significantly ($P \leq 0.05$) influence the peel firmness. Treatments 5 and 6 exhibited the least peel firmness of 5.90 N (23% reduction) and 4.10 N (46% reduction) on Day 21, respectively. The least reduction in the firmness was observed in fruit treated with anolyte water × 30s + HWT × 20s × 53°C + B13 (Treatment 1) and anolyte water × 30s + HWT × 30s × 53°C + B13 (Treatment 2) throughout the storage period. A minor increase in the peel firmness was observed for Treatments 1 (between Days 7 and 14) and Treatment 6 (between Days 0A and 7).

Table 7.5 Changes in the peel firmness (N) of *Penicillium digitatum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	7.66^{cd}	6.83^{bcd}	6.87^{bcd}	6.05^{abcd}	6.20^{bcd}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	7.66^{cd}	7.40^{bcd}	7.30^{bcd}	6.70^{bcd}	6.60^{bcd}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	7.66^{cd}	7.53^{cd}	7.57^{cd}	6.27^{bcd}	5.90^{abcd}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	7.66^{cd}	7.00^{bcd}	7.30^{bcd}	6.25^{bcd}	5.79^{abcd}
TW × 30s (5)	7.66^{cd}	7.13^{bcd}	6.90^{bcd}	5.77^{abcd}	5.47^{abc}
No treatment (6)	7.66^{cd}	6.92^{bcd}	6.65^{bcd}	5.23^{ab}	4.10^{a}
Significance					
Treatment (A)	*				
Storage Period (B)	*				
AB	*				
	16.1				

NS, *, ** Non-significant or significant at $P \leq 0.05$ or $P \leq 0.001$, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

Table 7.6 presents the changes in the peel firmness of fruit inoculated with *P. italicum*. The treatment, storage period and the interaction of both these factors were found to have

a significant (P≤0.001) influence on the peel firmness. A general decrease in the firmness was detected for all treatments. Treatments 5 and 6 exhibited the least firmness at the end of the storage period, of 5.65 N and 5.50 N, respectively. Fruit subjected to Treatment 1 demonstrated the least reduction in the peel firmness from 8.02 N to 7.40 N from Day 0B to Day 21. A minor increase in the peel firmness was observed for Treatments 1 (between Days 7 and 14) and Treatment 6 (between Days 0A and 7).

Table 7.6 Changes in the peel firmness (N) of *Penicillium italicum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	8.02^{hi}	8.10^{hi}	7.25^{efg}	7.45^{fg}	7.40^{fg}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	8.02^{hi}	7.15^{ef}	6.80^{de}	6.90^{def}	5.85^{ab}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	8.02^{hi}	8.00^{hi}	7.43^{fg}	6.35^{cd}	6.30^{cd}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	8.02^{hi}	7.80^{gh}	7.00^{def}	7.05^{ef}	6.87^{de}
TW × 30s (5)	8.02^{hi}	6.75^{de}	6.55^{cde}	6.05^{abc}	5.65^{a}
No treatment (6)	8.02^{hi}	6.85^{de}	7.00^{def}	6.10^{bc}	5.50^{a}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	**				
CV (%)	8.6				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

Diminishing fruit firmness is predominantly due to the action of pectic enzymes as the fruit matures (Muramatsu *et al*., 1996). The firmness of citrus fruit primarily depends on the rate of weight loss and turgidity (Hong *et al*., 2007). This trend is demonstrated in Tables 7.1 and 7.2 where Treatments 5 and 6 resulted in fruit with excessive weight loss, which can be correlated to Treatments 5 and 6 in Table 7.5 and 7.6 of fruit with the least peel firmness. Similarly, fruit subjected to Treatments 1 and 2 displayed the low PWL (Table 7.1) resulted in fruit with the least reduction in the peel firmness (Table 7.5). The fruit are more turgid, requiring a higher puncture force to penetrate the peel. Numerous studies have dealt with the correlation between citrus weight loss and firmness

(Muramatsu *et al*., 1996; Olmo *et al*., 2000, Singh and Reddy, 2006). Respiration rates immediately after hot water treatments rapidly increase (Hong *et al*., 2007). Transpiration may also occur through microscopic cracks on the fruit surface even though the fruit may appear sound (Rodov *et al*., 2000). Temperatures in excess of 56°C (30s) or 59°C (15s) can be detrimental to citrus and result in damage (Palma *et al*., 2013). However, in this study a temperature of 60°C did not cause any visual heat damage to the fruit peel nor was there a drastic reduction in the firmness.

An increase in the peel firmness during the storage can be attributed to either of two mechanisms; (1) the loss of moisture, leaving the peel hard and leathery (Ladaniya, 2008) or (2) endogenous calcium (Ca), which forms Ca-pectate from low methoxyl pectins produced through the heat-enhanced activity of pectinesterase (Sams *et al*., 1993). The cells would therefore have a higher resistance to enzymatic breakdown in addition the heat treatments have the ability to temporarily repair cracks and fissures on the surface, thus preventing the loss of moisture and promoting greater turgidity.

Based on the results, it can be deduced that *P. digitatum*- and *P. italicum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 20s × 53°C + B13 led to fruit of higher firmness at the end of the 21-day storage period.

7.6.4 Peel moisture content

Table 7.7 presents the changes in the peel moisture content (MC) of *P. digitatum*-inoculated kumquat fruit. The storage period had a significant influence on the MC at $P \leq 0.05$. However, the treatment and the interaction between the treatment and the storage period were found to not have a significant influence on the MC. Treatment 1 resulted in fruit with the highest MC of 58.3% on Day 21. Treatments 2, 3 and 4 results were not significantly different. As will be explained further in Section 7.6.5, the rate of increase in the TSS and the rate of reduction in the MC for *P. digitatum*-inoculated kumquat occurred more rapidly at the start of the storage period, compared to later on in the storage period.

Table 7.7 Changes in the moisture content (%) of *Penicillium digitatum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	59.8^{cd}	60.1^{cd}	60.4^{cd}	59.4^{cd}	58.3^{cd}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	59.8^{cd}	56.3^{bcd}	56.1^{bcd}	55.3^{bcd}	55.7^{bcd}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	59.8^{cd}	55.7^{bcd}	55.4^{bcd}	55.2^{bcd}	55.3^{bcd}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	59.8^{cd}	55.2^{bcd}	55.8^{bcd}	53.7^{bcd}	54.7^{bcd}
TW × 30s (5)	59.8^{cd}	57.6^{cd}	55.3^{bcd}	53.1^{bcd}	51.9^{bc}
No treatment (6)	59.8^{cd}	54.2^{bcd}	48.2^{abc}	46.8^{a}	45.5^{a}
Significance					
Treatment (A)	NS				
Storage Period (B)	*				
AB	NS				
CV (%)	11.3				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

Table 7.8 presents the changes in the peel MC of *P. italicum*-inoculated kumquat fruit. The treatment, storage period and the interaction of both these factors were found to significantly (P≤0.001) influence the change in the MC. All treatments demonstrated a decrease in the MC throughout the storage period. Treatment 6 produced fruit with the lowest MC at each sampling interval. Treatments 1 and 2 produced fruit with the least loss in moisture of 61.4% and 62.2% on Day 21, respectively. Fruit treated at a hot water temperature of 60°C (Treatments 3 and 4) developed a higher loss in moisture, compared to fruit treated at 53°C. Treatments 5 and 6 produced fruit with the greatest loss in MC in both Table 7.7 and 7.8.

Table 7.8 Changes in the peel moisture content (%) of *Penicillium italicum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	72.6^{fg}	70.2^{efg}	68.1^{ef}	64.3^{de}	61.4^{cde}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	72.6^{fg}	64.0^{de}	61.5^{cde}	62.6^{de}	62.2^{de}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	72.6^{fg}	68.8^{ef}	63.9^{de}	60.0^{cde}	58.2^{cd}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	72.6^{fg}	67.4^{ef}	68.4^{ef}	58.9^{cde}	58.0^{cd}
TW × 30s (5)	72.6^{fg}	66.8^{def}	60.8^{cde}	58.4^{cd}	53.1^{ab}
No treatment (6)	72.6^{fg}	58.3^{cd}	56.6^{bcd}	52.0^{a}	51.1^{a}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	**				
CV (%)	11.9				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

During storage the loss of moisture from the peel is continuously replenished by the movement of the moisture from the pulp. If this loss, due to the combined effect of respiration and transpiration, goes on unchecked, the fruit shrivels up and become unmarketable (Singh and Reddy, 2006). Over time, fractures may appear on the surface of fruit. These fractures can develop into invasion sites for pathogens. In addition, these fractures can increase tissue deterioration by enhancing the transpiration and shrinkage. Exposing the surface of the fruit to hot water treatments can result in physical changes to the epicuticular surface of the fruit by melting and redistributing the natural wax present on the fruit surface, thereby, sealing the fractures and cracks, which also extends to sealing of stomatal openings (Ben-Yehoshua, 2005).

Based on the results, it can be deduced that *P. digitatum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 20s × 53°C + B13 and *P. italicum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 30s × 53°C + B13 resulted in higher MC values at the end of the 21-day storage period.

7.6.5 Total soluble solids

The changes in the total soluble solids (TSS) of kumquat fruit inoculated with *P. digitatum* is presented in Table 7.9. The treatment and the interaction between the treatment and the storage period had a significance level of P≤0.05 while the storage period had a significance level of P≤0.001 with regard to the change in TSS. The TSS of fruit treated with Treatments 1, 2 and 3 were found to not be significantly different from each other on Day 21. These treatments resulted in fruit of lower TSS values than the other treatments. Treatment 6 resulted in the highest TSS of 13.8 °Brix by Day 21.

Table 7.9 Changes in the total soluble solids (°Brix) of *Penicillium digitatum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	8.3^{a}	9.3^{abc}	10.0^{bcd}	11.3^{efg}	11.2^{efg}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	8.3^{a}	8.9^{ab}	10.1^{bcde}	10.3^{cde}	11.4^{efg}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	8.3^{a}	9.2^{abc}	10.4^{cde}	11.0^{efg}	11.5^{efg}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	8.3^{a}	9.3^{abc}	10.5^{cde}	10.8^{ef}	11.9^{fg}
TW × 30s (5)	8.3^{a}	10.0^{bcd}	10.6^{def}	11.1^{efg}	12.9^{gh}
No treatment (6)	8.3^{a}	10.4^{cde}	10.8^{ef}	11.4^{efg}	13.8^{h}
Significance					
Treatment (A)	*				
Storage Period (B)	**				
AB	*				
CV (%)	11.0				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

The changes in the TSS of kumquat fruit inoculated with *P. italicum* is presented in Table 7.10. The storage period was found to have a significance level of P≤0.001 while the treatments and the combined effect of both the treatments and the storage period had a significance level of P≤0.05 with regard to the change in TSS. A general increase in the TSS was observed for all treatments. Treatments 5 and 6 displayed the greatest increase in the TSS of 12.55 °Brix and 12.90 °Brix on Day 21, respectively. Fruit treated with

anolyte water × 30s + HWT × 20s × 53°C + B13 (Treatment 1) and anolyte water × 30s + HWT × 30s × 53°C + B13 (Treatment 2) exhibited the least increase in TSS of 10.70 °Brix for both treatments. Treatments 5 and 6 gave rise to fruit with considerably higher TSS values. As presented in Table 7.8, Treatments 1 and 2 fruit had the highest MC values by Day 21. Similarly, these treatments resulted in fruit with the least increase in TSS. The rate of change in both the MC and TSS lessened toward the end of the storage period.

Table 7.10 Changes in the total soluble solids (°Brix) of *Penicillium italicum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	8.70^{a}	8.95^{ab}	10.40^{def}	10.60^{ef}	10.70^{efg}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	8.70^{a}	8.70^{a}	9.60^{cde}	10.13^{de}	10.70^{efg}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	8.70^{a}	9.05^{ab}	10.77^{efg}	10.43^{def}	11.18^{gh}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	8.70^{a}	9.20^{bc}	9.67^{cde}	11.25^{gh}	10.90^{fg}
TW × 30s (5)	8.70^{a}	9.50^{cd}	11.23^{gh}	12.30^{hi}	12.55^{hi}
No treatment (6)	8.70^{a}	9.50^{cd}	10.77^{efg}	12.58^{hi}	12.90^{hij}
Significance					
Treatment (A)	*				
Storage Period (B)	**				
AB	*				
CV (%)	8.4				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

The general increase in the TSS of citrus can be attributed to a loss in moisture, resulting in an increase in the concentration of soluble solids (D'hallewin *et al*., 1994; Olmo *et al*., 2000, Rodov *et al*., 2000, Ladaniya, 2008). This also serves as an important indicator of the internal quality of citrus fruit worldwide. By contrast, Hong *et al*. (2007) found that hot water treatments of 52°C for 2 minutes, 55°C for 1 minute and 60°C for 20 seconds did not significantly influence the TSS. Porat *et al*. (2000), Hong *et al*. (2007) and Garcia *et al*. (2016) also found that hot water did not affect the TSS levels in citrus fruit. A previous study by Kassim *et al*. (2016) found variation in the TSS levels of kumquat fruit due to different pre-packaging treatments of anolyte water, chlorinated water, hot water

and B13 (biocontrol agent). This study revealed that the combination of anolyte water, hot water and a biocontrol were effective in reducing the rate of change of the TSS of kumquat fruit. Kaewsuksaeng *et al*. (2015) found that lime fruit treated with hot water at 50°C for 5 minutes resulted in higher TSS values compared to 3 minutes. These values remained constant throughout the storage period, but control fruit that had been washed (Treatment 5) and fruit with no treatment (Treatment 6) resulted in an increase in the TSS values during storage.

Based on the results, it can be deduced that *P. digitatum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 20s × 53°C + B13 or anolyte water × 30s + HWT × 20s × 53°C + B13 or anolyte water × 30s + HWT × 20s × 60°C + B13 and *P. italicum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 20s × 53°C + B13 or anolyte water × 30s + HWT × 20s × 53°C + B13 developed lower TSS values at the end of the 21-day storage period.

7.6.6 Microbiological

This section presents and discusses the results for the total aerobic count, total coliform count and total fungal count on the surface of kumquat fruit.

7.6.6.1 Total aerobic plate count

Table 7.11 presents the influence of the different pre-packaging treatments on the aerobic plate count (APC) of *P. digitatum*-inoculated kumquat fruit. The treatment and the interaction between the treatment and the storage period had a significance level of $P \leq 0.05$ with regard to the APC. The storage period had a significantly higher influence on the APC at $P \leq 0.001$. Thereafter, the APC increased for all treatments until Day 21. Treatments 1-5 displayed a reduction in the APC values between Day 0B and 0A. Treatment 2 resulted in the lowest APC of 5.8 log $CFU.g^{-1}$ and 6.3 log $CFU.g^{-1}$ immediately after treatment (Day 0A) and on Day 21, respectively.

Table 7.11 Population dynamics of the total aerobic count (log CFU.g^{-1}) on the surface of *Penicillium digitatum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	6.0^{abc}	5.9^{ab}	6.1^{bc}	6.2^{cd}	6.4^{ef}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	6.0^{abc}	5.8^{a}	6.0^{abc}	6.1^{bc}	6.3^{de}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	6.0^{abc}	5.9^{ab}	6.1^{bc}	6.3^{de}	6.4^{ef}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	6.0^{abc}	5.9^{ab}	6.1^{bc}	6.3^{de}	6.5^{fg}
TW × 30s (5)	6.0^{abc}	6.0^{abc}	6.1^{bc}	6.4^{ef}	6.6^{gh}
No treatment (6)	6.0^{abc}	6.0^{abc}	6.2^{cd}	6.5^{fg}	6.7^{h}
Significance					
Treatment (A)	*				
Storage Period (B)	**				
AB	*				
CV (%)	2.3				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

Table 7.12 presents the influence of the different pre-packaging treatments on the APC of *P. italicum*-inoculated kumquat fruit. The storage period was found to significantly influence the APC at P≤0.001 level of significance. The treatment and the interaction between the treatment and storage period were found to have a less significant (P≤0.05) influence on the APC than the storage period. As with the *P. digitatum*-inoculated kumquat, the APC reduced between Day 0B and 0A for fruit from Treatments 1, 2, 3, 4, and 5. A reduction in the APC from Day 0B to Day 0A was noted for all treatments excluding the control (Treatment 6). Treatments 1, 2, and 3 caused the greatest reduction in the APC from Day 0B to Day 0A of 0.5 log CFU.g^{-1}. Thereafter, the APC increased for the remainder of the storage period. Treatment 2 resulted in the least APC of 6.3 log CFU.g^{-1} followed by Treatment 1 of 6.4 log CFU.g^{-1} by Day 21. Control samples demonstrated a continuous increase in the APC throughout the storage period.

Table 7.12 Population dynamics of the total aerobic count (log CFU.g^{-1}) on the surface of *Penicillium italicum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	6.1^{cd}	5.6^{a}	6.0^{c}	6.2^{de}	6.4^{ef}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	6.1^{cd}	5.6^{a}	6.1^{cd}	6.2^{de}	6.3^{e}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	6.1^{cd}	5.6^{a}	5.9^{bc}	6.4^{ef}	6.5^{efg}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	6.1^{cd}	5.7^{ab}	5.8^{abc}	6.3^{e}	6.5^{efg}
TW × 30s (5)	6.1^{cd}	5.8^{abc}	5.8^{abc}	6.5^{efg}	6.6^{fg}
No treatment (6)	6.1^{cd}	6.1^{cd}	6.3^{e}	6.6^{fg}	7.1^{h}
Significance					
Treatment (A)	*				
Storage Period (B)	**				
AB	*				
CV (%)	11.3				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

The aerobic bacterial load contamination can be attributed to a number of sources such as the environment (air and water) and during fruit handling and pre-harvest activities (Gultie and Sahile, 2013). Pao and Brown (1998) found a 1.8 log CFU.cm^{-2} reduction in the average APC of orange fruit by rinsing with potable water. Limited literature is available on the aerobic microbial load of kumquat fruit. This has thus been identified as a research gap, which this research aims to address.

Based on the results, it can be deduced that *P. digitatum*- and *P. italicum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 30s × 53°C + B13 developed the lowest APC values immediately after treatment on Day 0A and the end of the 21-day storage period.

7.6.6.2 Total coliform count

The changes in the total coliform count (CC) of *P. digitatum*-inoculated kumquat fruit are presented in Table 7.13. The treatment and storage period significantly (P≤0.001)

influenced the CC. The interaction between the treatment and the storage period were found to be less significant (P≤0.05) with regard to the change in the CC. Treatments 1, 3 and 5 were not significantly different from each other at 6.8 log CFU.g^{-1}. Treatment 1 had the greatest reduction in the CC of 0.9 log CFU.g^{-1} from Day 0B to 0A, whereas the least CC present on the fruit surface was as attributed to Treatment 2 (6.5 log CFU.g^{-1}) on Day 21.

Table 7.13 Population dynamics of the total coliform count (log CFU.g^{-1}) on the surface of *Penicillium digitatum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	**Storage Period (Days)**				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	6.5^{gh}	5.6^{a}	6.1^{cde}	6.3^{efg}	6.8^{jk}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	6.5^{gh}	5.9^{bc}	6.2^{def}	6.4^{fg}	6.5^{gh}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	6.5^{gh}	5.7^{ab}	6.1^{cde}	6.4^{fg}	6.8^{jk}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	6.5^{gh}	5.7^{ab}	6.0^{cd}	6.4^{fg}	6.7^{ij}
TW × 30s (5)	6.5^{gh}	6.5^{gh}	6.6^{hi}	6.6^{hi}	6.8^{jk}
No treatment (6)	6.5^{gh}	6.6^{hi}	6.7^{ij}	6.8^{jk}	7.2^{l}
Significance					
Treatment (A)	**				
Storage Period (B)	**				
AB	*				
CV (%)	2.9				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

The changes in the CC of *P. italicum*-inoculated kumquat fruit are presented in Table 7.14. The storage period was found to significantly (P≤0.001) influence the CC whereas the treatment and the interaction between both these factors were less significant (P≤0.05). After the application of Treatments 1 to 5, a notable decrease in the CC was observed on Day 0A, followed by an increase in the subsequent days until Day 21. Treatments 2, 3, 4, and 5 were not significantly different in efficacy at the end of the storage period (6.8 log CFU.g^{-1}). Treatment 2 caused the greatest reduction in the CC from Day 0B to 0A of 1.1 log CFU.g^{-1}. However, Treatment 1 produced fruit with the lowest CC of 6.7 log CFU.g^{-1} on Day 21.

Table 7.14 Population dynamics of the total coliform count (log CFU.g^{-1}) on the surface of *Penicillium italicum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	6.6^{g}	5.6^{b}	6.0^{cd}	6.5^{fg}	6.7^{gh}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	6.6^{g}	5.5^{a}	6.1^{de}	6.5^{fg}	6.8^{hi}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	6.6^{g}	5.6^{b}	6.1^{de}	6.7^{gh}	6.8^{hi}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	6.6^{g}	5.6^{b}	6.1^{de}	6.4^{f}	6.8^{hi}
TW × 30s (5)	6.6^{g}	6.4^{f}	6.5^{fg}	6.8^{hi}	6.8^{hi}
No treatment (6)	6.6^{g}	6.6^{g}	6.8^{hi}	7.0^{ij}	7.1^{j}
Significance					
Treatment (A)	*				
Storage Period (B)	**				
AB	*				
CV (%)	3.9				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

The presence of coliform bacteria on the surface of the fruit may have appeared due to handling by various parties during transport, temporary storage and during handling at any stage (National Advisory Committee on Microbiological Criteria for Foods, 1999). The presence of non-faecal coliforms such as *Enterobacter* spp. and *Klebsiella* spp. can be found in both soils and decaying vegetation, while *Escherichia coli* (*E. coli*) is associated with enteric microorganisms of a faecal nature. However, this study did not differentiate between the different types of coliform.

The Department of Health (2001) in South Africa specifies an acceptable maximum coliform count for raw fruit and vegetables to be 200 CFU.g^{-1}. Pao and Brown (1998) found a reduction in *E. coli* on orange fruit of up to 2.0 log cycles by merely washing and rinsing with potable water for 30 seconds. With the addition of a sanitizer, Pao *et al.* (2000) reduced *E. coli* on the surface of orange fruit by a drastic 0.9 to 3.5 log cycles. The immersion of oranges in hot water at 80°C for 1 min or 70°C for 2 min (5 log cycles) was more effective in reducing the population of *E. coli*, compare to chemical treatments (1.8 - 3.1 log cycles) (Pao and Davis, 1999). The reduction in the *E. coli* population from

these studies appear to be more than the reductions reported in Tables 7.13 and 7.14. This can be expected as the treatments and the type of citrus fruit differ. The author was unable to find published literature pertaining to the microbial load on kumquat fruit.

Based on the results for the CC, it can be deduced that *P. digitatum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 30s × 53°C + B13 and *P. italicum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 30s × 53°C + B13 carried the lowest CC immediately after treatment on Day 0A and the end of the 21-day storage period.

7.6.6.3 Total fungal count

Table 7.15 presents the change in the total fungal count of *P. digitatum*-inoculated kumquat fruit. The treatment, storage period and the interaction of both these factors were found to have a significant ($P \leq 0.05$) influence on the FC. A similar trend followed through from Table 7.11 to Table 7.16, where fruit treated with Treatments 1, 2, 3 and 4 exhibited a decreased in the microbiological count from Day 0B to Day 0A. Treatment 2 resulted in the greatest reduction of the FC from Day 0B to Day 0A of 0.3 log $CFU.mL^{-1}$. Treatments 1, 3, and 4 were not significantly different from each other at 6.7 log $CFU.mL^{-1}$. However, these were the lowest FC on Day 21. Sporulation and fungal formation were only observed on fruit without any treatment on Day 14.

Table 7.15 Population dynamics of the total fungal count (log CFU.g^{-1}) on the surface of *Penicillium digitatum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	6.1^{bcd}	5.9^{ab}	6.3^{de}	6.5^{ef}	6.5^{ef}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	6.1^{bcd}	5.8^{a}	6.4^{def}	6.6^{fg}	6.6^{fg}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	6.1^{bcd}	6.0^{abc}	6.2^{cd}	6.4^{def}	6.5^{ef}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	6.1^{bcd}	6.1^{bcd}	6.4^{def}	6.4^{def}	6.5^{ef}
TW × 30s (5)	6.1^{bcd}	6.1^{bcd}	6.5^{ef}	6.7^{gh}	6.8^{hi}
No treatment (6)	6.1^{bcd}	6.3^{de}	6.6^{fg}	6.8^{hi}	6.9^{i}
Significance					
Treatment (A)	*				
Storage Period (B)	*				
AB	*				
CV (%)	2.5				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

Table 7.15 presents the changes in the total fungal count (FC) of *P. italicum*-inoculated kumquat fruit. This count also includes the yeast present on the fruit surface. The treatment, storage period and the interaction of both these factors were found to have a significant (P≤0.05) influence on the FC. Treatment 4 reduced the FC from 5.9 log CFU.g^{-1} on Day 0B to 5.7 log CFU.g^{-1} on Day 0A. However, on Day 21 Treatment 2 produced fruit with the lowest FC of 6.6 log CFU.g^{-1}. No significant difference between Treatments 3 and 4 were noted. Sporulation and fungal formation were observed only on the control treatment by Day 14.

Table 7.16 Population dynamics of the total fungal count (log CFU.g^{-1}) on the surface of *Penicillium italicum*-inoculated kumquat fruit over a 21-day storage period subjected to different integrated pre-packaging treatments

Treatment	Storage Period (Days)				
	0B	**0A**	**7**	**14**	**21**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	5.9^{c}	5.7^{ab}	5.9^{c}	6.6^{fg}	6.8^{gh}
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	5.9^{c}	5.7^{ab}	5.9^{c}	6.5^{f}	6.6^{fg}
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	5.9^{c}	5.7^{ab}	5.7^{ab}	6.6^{fg}	6.7^{g}
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	5.9^{c}	5.6^{a}	5.6^{a}	6.6^{fg}	6.7^{g}
TW × 30s (5)	5.9^{c}	5.8^{bc}	6.0^{cd}	6.7^{g}	6.9^{hi}
No treatment (6)	5.9^{c}	6.0^{cd}	6.1^{de}	6.8^{gh}	7.0^{i}
Significance					
Treatment (A)	*				
Storage Period (B)	*				
AB	*				
CV (%)	4.5				

NS, *, ** Non-significant or significant at P≤0.05 or P≤0.001, respectively. Means within a column followed by the same letter(s) are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05), (n=3). CV, Coefficient of variation; 0B, Day 0 before treatment application; 0A, Day 0 after treatment application; HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets

Due to the manner in which the kumquat is consumed, it is of special concern to determine the microbial load on the fruit surface as consumption of contaminated fruit can pose a food safety threat and hazard to human health (Gultie and Sahile, 2013). Enumeration of aerobic microorganisms is a common indicator of the quality and probable shelf life of raw fresh foods (Stannard, 1997). However, yeast or mould counts are more relevant indicators for commodities of low pH.

In addition to the beneficial effect of heat treatments on kumquat fruit, anolyte water may further improve the microbial quality of fruit (Palma *et al*., 2013; Kassim *et al*., 2016). Chapters 4 and 5 clearly indicated that anolyte water possesses sufficient free chlorine to reduce fungal growth. The active compound of anolyte water is the hypchlorous acid (HOCL) (Acher *et al*., 1997; Whangchai *et al*., 2010). The HOCL is capable of oxidising microbial cell nucleic acid and proteins, thereby lethally damaging the cells. Kim *et al*. (2000) and Riondet *et al*. (2000) suggested that the oxidation-reduction potential (ORP) was also a contributor toward microbial inactivation due to changes in the metabolic fluxes and ATP production. The addition of a third treatment of a biocontrol agent

supplemented the germicidal action of the anolyte water and hot water in a preventative manner. The inability of the biocontrol agent to provide a curative treatment is due to this antagonist being unable to penetrate the fruit tissue to the location of the pathogen (Abraham *et al*., 2010). Therefore, each treatment has a specific mode of action as a disinfectant, curative and preventative.

Many fruits are capable of withstanding exposure to hot water temperatures of 50–60°C for up to 10 min (Lurie, 1998). However, the exposure times and associated temperatures required to kill those bacterial cells, which are commonly associated with foods, are in excess of 10 minutes and 50–60°C, respectively. *E. coli* requires a treatment time of 20-30 minutes at 57.3°C and *Streptococcus thermophiles* requires an exposure time and temperature of 70-75°C (Thakur *et al*., 2000). Hsu and Beuchat (2012) stated that bacteria do not play as an important role in the spoilage of fruit as moulds and yeasts, which are capable of inducing appreciable spoilage of fruit. This is due to the inherent acidity associated with many fruit as well as the presence of bactericidal substances that are able to destroy certain kinds of bacteria. However, once ingested, bacteria can have detrimental effects on human and animal health (Leff and Fierer, 2013). Therefore, it is of importance to determine the effects of the combined treatments identified in this study on the total aerobic and coliform counts in addition to the more dominant *P. digitatum* and *P. italicum* fungal pathogens affecting citrus fruit.

According to Tables 7.11 to 7.16, there was not a drastic reduction in the APC, CC and *P. digitatum* and *P. italicum* counts between days 0B and 0A. This could be attributed to the spore cells remaining dormant on the fruit surface due to the applied treatments. However, once the slurries, which were obtained from the fruit surface (Section 7.4.6), were plated onto the nutrient-rich media, the spores began to sporulate giving rise to large microbial population numbers. However, a reduction in the population for APC, CC and *P. digitatum* and *P. italicum* was observed between Day 0B and 0A, alluding to the effectiveness of the combined treatments as anolyte water only and hot water only are not sufficient to eradicate these microorganisms.

Based on the results, it can be deduced that Treatment 2, anolyte water × 30s + HWT × 30s × 53°C + B13, resulted in the least FC present on the fruit surface on Day 0A for *P. digitatum*-inoculated kumquat fruit. Treatments 1, 3 and 4 led to the lowest FC at the end

of the 21-day storage period. For *P. italicum*-inoculated kumquat fruit anolyte water × 30s + HWT × 30s × 53°C + B13 developed the least FC immediately after treatment on Day 0A and the end of the 21-day storage period.

7.6.7 Subjective quality analysis

P. digitatum- and *P. italicum*-inoculated kumquat fruit subjected to Treatments 1 to 4 remained sound during the 21 days of storage and were allocated a value of '6', representative of excellent on the hedonic scale. This is of great importance as the required shipping time from South Africa to Europe is 14 days (Steyn, 2016). This subjective quality analysis reveals that an additional seven days of shipping would not have a detrimental effect on the visual quality of the kumquat when treated. No visual formation of fungal infection or softening was observed for fruit treated with (1) anolyte water × 30s + HWT × 20s × 53°C + B13; (2) anolyte water × 30s + HWT × 30s × 53°C + B13; (3) anolyte water × 30s + HWT × 20s × 60°C + B13 or (4) anolyte water × 30s + HWT × 30s × 60°C + B13. In addition, these treatments resulted in fruit with slightly shinier surfaces, compared to Treatments 5 and 6. This could be attributed to the melting and redistributing of the epicuticular wax present on the fruit, forming a more even, smooth and shiny appearance (Palma *et al*., 2013). Fruit treated with TW × 30s began to soften by Day 14 for both *P. digitatum*- and *P. italicum*-inoculated kumquat fruit. Fruit with no treatment (Treatment 6) displayed excessive moisture loss, softening and shrivelling by Day 21, with visible signs of fungal infection in both *P. digitatum*- and *P. italicum*-inoculated samples. The results obtained for the *P. digitatum*- and *P. italicum*-inoculated kumquat fruit were observed to be similar. Table 7.17 presents the subjective rating and description of *P. digitatum*- and *P. italicum*-inoculated kumquat fruit on Day 21.

Table 7.17 Subjective quality analysis of *Penicillium digitatum*- and *Penicillium italicum*-inoculated kumquat fruit on Day 21 subjected to different integrated pre-packaging treatments

Treatment	Rating (Day 21)	Description
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	6	Shiny and smooth peel Slight wrinkling by Day 21 No visible mould formation
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	6	Shiny and smooth peel Slight wrinkling by Day 21 No visible mould formation
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	6	Shiny and smooth peel Slight wrinkling by Day 21 No visible mould formation No visible heat damage
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	6	Shiny and smooth peel Slight wrinkling by Day 21 No visible mould formation No visible heat damage
TW × 30s (5)	5	Slight wrinkling by Day 21 No visible mould formation
No treatment (6)	2	Softening of the peel by Day 21 Slight wrinkling of the peel by Day 14 Visible mould formation

HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

7.6.8 Overall ranking of fruit

The overall ranking of the fruit integrated a holistic view of the effects of each of the six treatments on the PWL, peel colour, peel firmness, MC, TSS, APC, CC, FC and the subjective quality analysis. Tables 7.18 and 7.19 represent the scores allocated to each of the treatments for *P. digitatum*- and *P. italicum*-inoculated kumquat fruit. In both cases Treatment 1, anolyte water × 30s + HWT × 20s × 53°C + B13, and Treatment 2, anolyte water × 30s + HWT × 30s × 53°C + B13, obtained higher ranks of 12 and 15 for *P. digitatum*-inoculated fruit (Table 7.18) and 14 and 13 for *P. italicum*-inoculated fruit (Table 7.19), respectively. This indicates that these treatments were the most effective in maintaining the quality of the kumquat fruit and reducing microbial growth. On the contrary, Treatment 6 resulted in the highest final rank in Tables 7.18 and 7.19, which

indicated a poor effect on the quality of the fruit as expected. These results indicate the beneficial effect of combining anolyte water for 30 s, hot water at 53°C for 20 s and B13 biocontrol on the physical, chemical and microbiological quality of kumquat fruit.

Based on the results, it can be deduced that *P. digitatum*-inoculated kumquat fruit treated with anolyte water × 30s + HWT × 20s × 53°C + B13 led to fruit of an overall better quality throughout the 21-day storage period. While and *P. italicum*-inoculated fruit treated with anolyte water × 30s + HWT × 30s × 53°C + B13 displayed slightly better quality than the other treatments.

Table 7.18 The overall ranking based on the effectiveness of each treatment on the quality parameters for *Penicillium digitatum*-inoculated kumquat fruit

Penicillium digitatum										
Treatment	**PWL**	**Peel Colour**	**Peel Firmness**	**MC**	**TSS**	**APC**	**CC**	**FC**	**Subjective Quality Analysis**	**Total**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	1	2	1	1	1	2	1	2	1	**12**
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	2	3	1	2	1	1	3	1	1	**15**
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	4	2	2	2	1	2	2	3	1	**19**
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	4	1	2	2	2	2	2	4	1	**20**
TW × 30s (5)	3	3	3	3	3	3	4	4	2	**28**
No treatment (6)	5	4	4	4	4	3	5	5	3	**37**

HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

Table 7.19 The overall ranking based on the effectiveness of each treatment on the quality parameters for *Penicillium italicum*-inoculated kumquat fruit

Penicillium italicum										
Treatment	**PWL**	**Peel Colour**	**Peel Firmness**	**MC**	**TSS**	**APC**	**CC**	**FC**	**Subjective Quality Analysis**	**Total**
Anolyte water × 30s + HWT × 20s × 53°C + B13 (1)	2	2	1	2	1	1	2	2	1	**14**
Anolyte water × 30s + HWT × 30s × 53°C + B13 (2)	1	1	4	1	1	1	1	2	1	**13**
Anolyte water × 30s + HWT × 20s × 60°C + B13 (3)	2	1	3	3	3	1	2	2	1	**18**
Anolyte water × 30s + HWT × 30s × 60°C + B13 (4)	2	1	2	3	2	2	2	1	1	**16**
TW × 30s (5)	3	3	5	4	4	3	3	3	2	**30**
No treatment (6)	4	4	6	5	5	4	4	4	3	**39**

HWT, hot water treatment; TW, tap water; s, dipping time in seconds; +, 'combined with'. Each treatment was allocated a treatment number within brackets.

7.7 Conclusion

The results of this study substantiated the findings in previous Chapters 4, 5 and 6. The integrated application of anolyte water, hot water and B13 on kumquat fruit were most effective in preventing decay due to *P. digitatum* and *P. italicum* and maintaining fruit quality. The fruit could be stored for an additional seven days at 4.5°C when treated, which is the prescribed shipping temperature. The aim of this study was to determine the treatment that had the most beneficial effect on the physical, chemical, microbiological and subjective qualities in treated kumquat fruit.

The treatment combinations of anolyte water × 30s + HWT × 20s × 53°C + B13 (Treatment 1) and anolyte water × 30s + HWT × 30s × 53°C + B13 (Treatment 2) performed optimally for both *P. digitatum*- and *P. italicum*-inoculated kumquat fruit. Treatments, storage period and the interaction of these factors had a significant influence on the PWL, hue angle, peel firmness, MC, TSS, APC, CC and FC at either $P \leq 0.05$ or $P \leq 0.001$. fruit without any treatments displayed the poorest quality. Treatment 1 produced fruit of the best quality for fruit inoculated with *P. digitatum* while Treatment 2 was more beneficial for *P. digitatum*-inoculated fruit.

Treatment 1 fruit samples exhibited a low PWL (37.87%), firm fruit (6.20 N), high MC (58.3%), low TSS (11.2 °Brix) by Day 21 and a low CC (5.6 log $CFU.g^{-1}$) immediately after treatment. Whereas Treatment 2 resulted in fruit of low PWL (27.72%), slower colour change (70.92°), low MC (62.2%), low TSS (10.7 °Brix) on Day 21 and a low CC (5.5 log $CFU.g^{-1}$) and FC (5.7 log $CFU.g^{-1}$) immediately after treatment. Visible mould formation was observed only on control samples on Day 14. Treatment 2 only slightly outperformed Treatment 1 with regard to the overall quality of the fruit for *P. italicum*-inoculated fruit. However, Treatment 1 outperformed Treatment 2 for *P. digitatum*-inoculated fruit. It is, therefore, recommended that Treatment 1 be applied using the IPCTU on kumquat fruit as *P. digitatum* is the more prevalent pathogen infecting citrus fruit.

Anolyte water, hot water and B13 combined to provide enhanced quality of the kumquat fruit due to their modes of action. Anolyte water is an environmentally friendly treatment generated by the electrolysis of salt and water with the active compound being hypochlorous acid, which has strong disinfecting capabilities (Whangchai *et al*., 2010). An initial disinfection of fruit with anolyte water for Treatments 1 to 5 ensures that existing surface pathogens are removed. This is demonstrated by a decrease in the APC, FC and CC for both *P. digitatum*- and *P. italicum*-inoculated kumquat fruit immediately after treatment (Day 0B to Day 0A). Thereafter, the fruit were treated with hot water at 53°C for either 20s (Treatments 1 and 3) or 30s (Treatments 2 and 4). The curative effect of the hot water is in its ability to induce plant host resistance to pathogenic infections (Ben-Yehoshua *et al*., 2005). In addition, the heat smoothen the surface of the fruit by melting and redistributing the epicuticular wax (Hong *et al*., 2007). This causes the sealing of surface cracks and fissures preventing the loss of moisture and eliminating entry points for pathogens. The third effect offered by the B13 biocontrol agent is the prevention of future infections (Abraham *et al*., 2010).

7.8 References

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8. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The overall goal of this study was to design, construct and evaluate an integrated postharvest citrus treatment unit, specific for kumquat fruit and capable of operation in the orchard. This addresses the challenge of having the fruit transported to a packhouse located a distance from the point of harvest. The delay between fruit harvesting and processing will be reduced and fruit treated earlier. Thus, removing the prevailing field heat, which consequentially will reduce decay caused by uncontrolled pathogens. *Penicillium digitatum* and *P. italicum* have been identified as the main postharvest pathogens affecting kumquat fruit, resulting in excessive postharvest losses. Therefore, the pre-packaging treatments used in this study have been based on: (1) reducing the rate of decay as a result of the *Penicillium* spp. pathogen, (2) being environmentally and consumer friendly, and (3) having a beneficial effect on the postharvest quality of kumquat fruit. There exists a dearth of information regarding the integrated use of anolyte water, hot water and a yeast biocontrol agent as pre-packaging treatments. More importantly, the novelty of this research is the method in which these treatments are applied to kumquat fruit.

The physical properties of kumquat, orange, grapefruit and lemons were determined to optimally design the pre-packaging treatment unit, as indicated in Chapter 3. The mass and dimensions of the fruit are critical parameters in the design process. The mean mass for the kumquat, orange, grapefruit and lemon samples were found to be 15.68 g, 340.84 g, 374.13 g and 130.00 g, respectively. Kumquat and lemons were found to have an oval shape, oranges were defined as being round to slightly oblate and grapefruit as being oblate. The fruit shape can have an effect on the manner in which the fruit are conveyed and treated in processing equipment. The fruit shape also influences the rate of heat transfer during thermal treatment, where larger fruit will require a longer time to reach the desired temperature. However, the thermal properties of fruit are not within the scope of this study.

The *in vitro* and *in vivo* study revealed that neither the anolyte water nor chlorinated water inhibited growth of the yeast B13 biocontrol agent (Chapter 4). Anolyte water combined with B13 was the most effective in reducing the proliferation of *P. digitatum*, whereas B13 only, and chlorinated water combined with B13 were effective in reducing the proliferation of *P. italicum*. Anolyte water only and anolyte water combined with B13 resulted in more aesthetically pleasing fruit, which appeared shiny and smooth. Further research was required to ascertain which of anolyte water or chlorinated water was a stronger disinfectant of kumquat fruit. Additionally, the effect of the integrated application of anolyte water or chlorinated water combined with hot water and the yeast biocontrol agent, B13 (*Candida fermentati*), compared to individually applied treatments on the postharvest physical, chemical and microbiological quality of kumquat fruit was necessary. This prompted the experiment contained in Chapter 5, which revealed that integrated treatments were more effective than individual treatments of kumquat fruit on a laboratory scale. Anolyte water was a more effective disinfectant, compared to chlorinated water. The use of anolyte water (100 $mg.kg^{-1}$ at ambient temperature) combined with hot water (53°C for 20 seconds) and B13 had a beneficial effect on the decay severity, PWL, firmness, MC and TSS. The storage conditions were found to be highly significant ($P \leq 0.001$) for all quality parameters. While, the treatments had significantly influenced ($P \leq 0.001$) the decay severity, PWL, peel firmness, peel MC and TSS. Fruit treated with (1) anolyte water, hot water and B13 and (2) anolyte water and hot water did not exhibit any decay throughout the storage period. Treatments that included anolyte water resulted in lower PWL values and higher MC values for both *P. digitatum*- and *P. italicum*-inoculated fruit. Based on the results it can be recommended that the integrated treatments were more effective in reducing decay and maintaining the fruit quality, compared to individual treatments.

Chapter 6 demonstrated the application of the postharvest citrus treatment unit, which was designed and constructed based on the results of Chapters 4 and 5. The three main pre-packaging treatments of the unit are: (1) anolyte water – disinfection, (2) hot water treatment – curative, and (3) a yeast B13 biocontrol agent - preventative. The power consumptions for Tank D (hot water) and Tank F (biocontrol) was 4.13 kW and 2.08 kW, respectively. Glasswool insulation was installed around Tank D to prevent the loss of

energy to the surroundings. The insulation resulted in an energy saving of 243.50 J, compared to if no insulation was used. The thermal efficiency of Tank D was 72% and Tank F was found to be 87% efficient. A payback period of 0.91 years and carbon ratio of 0.46 kg CO_2.day^{-1} was calculated. The tank operated outdoors under non-laboratory conditions. Due to financial constraints, the unit was unable to be operated in a kumquat orchard. However, the IPCTU operated successfully during the experiment. Based on the operation, economic and thermal analysis, this unit can be recommended for use by small-scale farmers.

Chapter 7 focused on determining the most effective treatment for kumquat fruit, which can be applied to industry. Treatments, storage period and the interaction of these factors had a significant influence on the PWL, hue angle, peel firmness, MC, TSS, APC, CC and FC at either $P \leq 0.05$ or $P \leq 0.001$. The treatment combinations of (1) anolyte water × 30s + HWT × 20s × 53°C + B13 and (2) anolyte water × 30s + HWT × 30s × 53°C + B13 performed optimally for both *P. digitatum*- and *P. italicum*-inoculated kumquat fruit. Treatment 1 fruit samples exhibited a low PWL (37.87%), firm fruit (6.20 N), high MC (58.3%), low TSS (11.2 °Brix) by Day 21 and a low CC (5.6 log CFU.g^{-1}) immediately after treatment. Whereas, Treatment 2 fruit displayed low PWL (27.72%), slower colour change (70.92°), low MC (62.2%), low TSS (10.7 °Brix) on Day 21 and a low CC (5.5 log CFU.g^{-1}) and FC (5.7 log CFU.g^{-1}) immediately after treatment. Visible mould formation was observed only on control samples on Day 14. Therefore, it is recommended that Treatment 1 (anolyte water × 30s + HWT × 20s × 53°C + B13) is the most effective integrated treatment of kumquat fruit using the postharvest citrus treatment unit.

8.1 Future Research and Recommendations

It is expected that ongoing research will be conduct on the unit in terms of modifications and testing it on other citrus such as lemons, and even on other horticultural commodities. The unit is predominantly manually operated with the exception of the electric hoist, which controls the conveyance of the fruit between treatments.

Some of the modifications and recommendations relating to the prototype are as follows:

1. To automate the system by using a Raspberry Pi (Raspberry Pi Foundation, UK Registered Charity 1129409), which is a compact yet fully customizable and programmable computer with multiple applications.
2. Anolyte water, hot water and biocontrol tanks can be replaced with a fully functional conveyor and spray system with nozzles to apply the treatments.
3. The modification of a dry container to house the unit and assist in transportation.
4. Implementing renewable energy methods as a source of power for operation of the unit such as solar panels.

Future research opportunities:

1. A detailed microbial analysis at various stages of the kumquat fruit supply chain will be supportive in identifying the sources and points of contamination.
2. A quantification of the losses in horticultural commodities due to time delays and temperatures from the point of harvest to pre-packaging treatments.
3. To further investigate the use of the prototype in orchards.

8.2 Practical Relevance

This research study addresses the following practical issues relating to citrus fruit:

1. The implementation of environmentally and consumer friendly treatments to address the challenge of *P. digitatum* and *P. italicum* decay of kumquat fruit.
2. The unit was constructed from easily sourced and inexpensive materials.
3. Anolyte water and the biocontrol agent can be purchased locally in South Africa and the hot water can be generated on site.
4. A shelf life extension of seven days during transport at 4.5°C – from 14 to 21 days was achieved.
5. The pre-packaging treatments of kumquat fruit was optimised.
6. There is now a greater understanding of the postharvest handling of kumquat fruit.
7. This treatment principle can be applied to other horticultural commodities.
8. The implementation of the unit for small-scale farmers could reduce their lack of access to large packhouses.

9. Small-scale farmers can now become self-reliant, creating investments in terms of human resources and job creation.
10. The unit has also been used for other projects at the University of KwaZulu-Natal, thereby promoting further research.

It is anticipated that the findings of this study will be applied to optimise the postharvest handling of kumquat fruit in South Africa for both local and export markets.

9. APPENDIX A – PACKHOUSE TREATMENTS

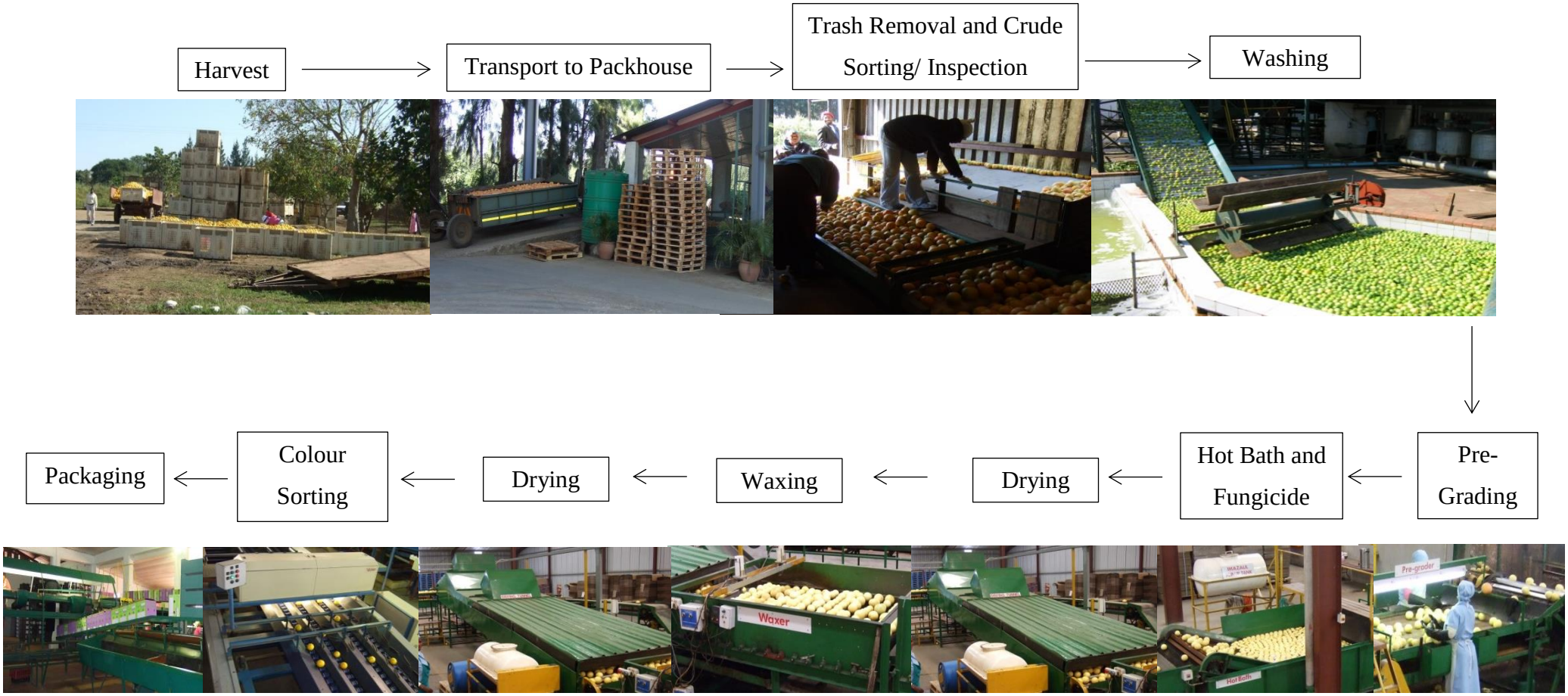

Figure 9.1 General citrus processing diagram (after Tugwell, 1988; Di Giacomo, 2002)

Table 9.1 Summary of diseases and physiological disorders of citrus fruit

Disease Classification	Citrus Cultivar	Disease/ Physiological Disorder	Symptoms	Additional Information	Prevention/ Remedy/ Control	Reference
Pre-harvest						
Bacterial disease	All citrus cultivars but may differ in the degree of susceptibility	Asiatic citrus canker caused by *Xanthomonas axonopodis* pv.*citri* (Xac A)	Raised lesion appearing on leaves, corky/ scab-like lesions on the fruit, premature fruit drop and poor fruit quality	Affects citrus trees. Areas that are susceptible experience high rainfall and humidity	Plants own defense mechanism, cultural practices, such as wind breaks, copper sprays	Stall (1988); Khalaf *et al*. (2007)
Fungal Disease in Nursery and Orchards (affecting the fruit)	All citrus, mainly affecting orange, mandarin, lemon, and grapefruit	Black spot caused by *Guignardia citricarpa* (Kiely)	Premature fruit abscission. The four catagories of symptoms are (1) hard spot, (2) freckle spot, (3) virulent or spreading, and (4) false melanose or speckled blotch	Symptoms may appear during late stages fruit development or after harvest. Symptoms vary among cultivars	Removal of infected trees and fruit from orchards, copper fungicides, spore trapping, fruit maintained at below 20°C after harvest	Kotze (1988); Korf *et al*. (2001); Bonants *et al*. (2003); Yonowa *et al*. (2013)
Postharvest						
Postharvest fungal disease	All citrus	Blue mould caused by *Penicillium italicum* Wehmer	Diseased tissue appears to be soft, watery and discoloured. Formation of a white powdery growth forms on lesions and develops into a mass of blue spores	Healthy fruit can be infected due to the movement of spores	Application of synthetic fungicides, hot water treatment, sodium carbonate, and sodium bicarbonate	Brown and Eckert (1988a); Palou *et al.* (2002); Venditti *et al*. (2005)
Postharvest fungal disease	All citrus	Green mould caused by *Penicillium digitatum* (Pers.:Fr.) Sacc.	The initial symptoms are similar to that of blue mould. The fruit becomes enveloped in a mass of olive green spores	Wounding during harvesting and postharvest handling initiates the action of this pathogen. Healthy fruit can be infected due	Application of synthetic fungicides, hot water treatment, sodium carbonate, and sodium bicarbonate	Brown and Eckert (1988b); Smilanick *et al*. (1997); Smilanick *et al*. (1999); Pavoncello *et al*. (2001); Palou *et al*.

Disease Classification	Citrus Cultivar	Disease/ Physiological Disorder	Symptoms	Additional Information	Prevention/ Remedy/ Control	Reference
				to the movement of spores		(2002); Venditti *et al*., 2005, Youssef *et al*. (2014)
Postharvest fungal disease	All citrus, particularly	*Geotrichum candidum*	Light to dark yellow water-soaked, raised lesions. White or cream mycelium may appear	Sour rot is stimulated by the presence of green mould	Preventing fruit contact with the soil during harvest. Delayed harvesting till later in the day. Minimizing fruit storage temperatures	Merciera and Smilanick, 2005; Smilanick *et al*., 2005; Ladaniya, 2008; Talibi *et al*., 2012
Postharvest fungal disease	All citrus	Stem-end rot caused by *Diplodia natalensis* P. Evans	The fungus starts at the stem and penetrates the rind and core. Decay is uneven and resembles finger-like projections of brown tissue. Mycelium form at the advanced stage of infection	Citrus that have been degreened using ethylene (5-10µl.L^{1}) are particularly susceptible. Temperatures in excess of 21°C promote fungal growth	The use of fungicides before and after degreening. Immediate cooling after packing	Brown (1986); Brown and Eckert (1988c); Brown and Lee (1993); Zhang and Swingle (2005)
Rind disorders	All cultivars, but mainly grapefruit, lemons and lime	Rind disorder caused by chilling injury	Browning of the flavedo, albedo and dark, sunken tissue	Chilling injury is as a result of exposing the fruit to too low temperatures before and/or after harvest	Heat treatments, intermittent warming, temperature conditioning, application of a wax, modified atmosphere packaging	Wardowski (1988a); Porat *et al*. (2004); Sapitnitskaya *et al*. (2006)
Stem-end rind breakdown	All cultivars but mainly in oranges	Rind disorder caused by aging.	Darkening and collapsing of the rind around the stem-end	Can result from an imbalance in potassium and nitrogen. Stem-end breakdown is associated with moisture loss and occurs mainly in thin-skinned small fruit. Symptoms usually occur	Maintaining high humidity environments, harvested fruit should be protected against heat and water loss, which can be achieved by use of a wax	Grierson (1986); Wardowski (1988b); Ritenour *et al*. (2004)

Disease Classification	Citrus Cultivar	Disease/ Physiological Disorder	Symptoms	Additional Information	Prevention/ Remedy/ Control	Reference
				two to seven days after packing		

Table 9.2 The effects of different heat treatments applied to citrus fruit

Type of Treatment	Exposure Time	Exposure Temperature	Fruit	Effect	Reference
Thermal curing	3 days	36°C	Eureka lemons	Prevention of *Penicillium* decay for > 2 months at 17°C	Kim *et al*. (1991)
				Production of scoparone	
Hot air	3 hours	48°C	Marsh grapefruit	Maintained fruit market quality	McGuire and Reeder (1992)
	2 hours	49°C			
Hot Water	-	53°C	Kumquat	Improved fruit appearance, reduced weight loss and rot development	Schirra *et al*. (1995)
Hot water	120 seconds or 30 seconds	53°C or 56°C	Kumquat	Reduction in blue and green mould	Ben-Yehoshua *et al*. (2000)
Hot water (dipping)	120 seconds	52°C	Oroblanco	Reduced fruit softening and button abscission.	Rodov *et al.* (2000)
				Inhibited yellow colour formation in combination with individual polyethylene packaging	
Hot drench brushing	10 seconds	60°C	Oroblanco	Reduced fruit softening and button abscission.	Rodov *et al.* (2000)
				Delayed colour change	
Hot water (dipping)	120 seconds	53°C	Kumquat	Reduced decay	Rodov *et al.* (2000)
				Reduced weight loss	
Hot water (rinsing)	20 seconds	62°C	Star Ruby Grapefruit	Reduced chilling injury by 85% after 8 weeks	Sapitnitskaya *et al*. (2006)
Hot water dipping	120 seconds	50°C	Kumquat	Maintained 'fresh' appearance, reduced decay, reduced weight loss, maintained quality traits	Schirra *et al*. (2011)
Hot air	30 hours	37°C	Kumquat	Loss of exterior gloss, excessive weight loss, diminished fruit quality	Schirra *et al*. (2011)
Hot water dipping	20 seconds	56°C	Tarocco oranges	Reduced weight loss, inhibition of green mould spore germination, maintained internal and external quality traits	Strano *et al*. (2014)
Hot water dipping	180 seconds	52°C	Tarocco oranges	Increased levels of alcohols, esters and aliphatic aldehydes	Strano *et al*. (2014)

'-', Information not provided in the research source.

Table 9.3 The effects of different surface coatings applied to citrus fruit

Description of Coating	Fruit	Effect	Reference
Beeswax emulsion and TAL Pro-long	Pineapple orange	Improved fresh orange juice volatiles and flavour	Nisperos-Carriedo *et al*. (1990)
Patented edible composite coating	Mature oranges	Improved volatiles and flavour	Nisperos-Carriedo *et al*. (1991)
Citral (120 second dipping time)	Mature light green lemons	Significantly reduced decay	Ben-Yehoshua *et al*. (1992)
		Fruit dipped in 1% citral resulted in phytotoxic damage	
Low molecular weight chitosan (0.1% and 0.2%)	Murcott tangor	Improved firmness, TTA, TSS, ascorbic acid, reduced water loss	Chien *et al*. (2007)
		Reduced postharvest decay (blue and green mould)	
Chitosan and $CaCl_2$ complex	Kumquats	Delay in ripening and senescence	Li *et al*. (2008)
Imazalil (3000 $mg.L^{-1}$) supplemented polyethylene wax	Navel oranges	Shiny fruit but resulted in off-flavours, compared to uncoated fruit	Njombolwana *et al*. (2013)
		Higher weight loss and less firm fruit, compared to carnauba wax supplemented with imazalil	
Carboxymethyl cellulose (1.5% w/v)	Rishon and Michal mandarins	Improved firmness, reduced weight loss and a glossy exterior	Arnon *et al*. (2014)

Table 9.4 The effects of different UV irradiation intensities on citrus fruit

UV Irradiation Intensity	Fruit	Effect	Reference
5.0 $kJ.m^{-2}$	Lemon	Increased production of scoparone	Ben-Yehoshua *et al*. (1992)
		Reduced green mould	
1.5 $kJ.m^{-2}$	Kumquat	Increased production of scoparone	Rodov *et al*. (1992)
		Reduced green mould	
2.2 $kJ.m^{-2}$	Marsh grapefruit	Reduced the incidence of green mould to 14%	Stevens *et al*. (1996)
1.3 $kJ.m^{-2}$	Dancy tangerines	10-fold reduction in the onset of green mould	
3.2 $kJ.m^{-2}$	Mature grapefruit	Reduced decay from 72% to 16 %	Lers *et al*. (1998)
3.0 $kJ.m^{-2}$	Washington Navel orange	Significant decay reduction in late harvested fruit	D'hallewin *et al*. (1999)
	Biondo Comune orange	Significant decay reduction in late harvested fruit	
0.5 $kJ.m^{-2}$ of UV-C	Star Ruby Grapefruit	Reduced decay caused by green mould to 2-3%	D'hallewin *et al*. (2000)
>0.5 $kJ.m^{-2}$ of UV-C		Higher doses resulted in tissue necrosis and peel browning	
		Fruit harvested earlier (less mature) exhibited more severe damage	
7.28 and 15.66 $kJ.m^{-2}$ of UV-C	Valencia oranges	Did not effectively control citrus black spot. However, the appearance of quiescent black spot lesions were reduced	Canale *et al*. (2011)

Table 9.5 The effects of different hypochlorite concentrations applied to citrus fruit

* Hypochlorite Concentration	Exposure Time	Fruit	Effect	Reference
200-250 ppm and pH 6.0-7.5 (10% strength sodium hypochorite)	120 seconds	Kumquat	Reduced decay	Hall (1986)
150 $mg.l^{-1}$ active chlorine, pH 8	60 seconds	Lemons	Hypochlorite treatment alone resulted in higher decay rates	Stange and Eckert (1994)
100 $\mu g.ml^{-1}$ free chlorine	120 seconds	Satsuma mandarin	Significant reduction in decay. Positive influence on the b* component colour	Sen *et al*. (2007)
1000 ppm	120 seconds	Nagpur mandarins	Reduced decay for 30 days at ambient conditions	Ladaniya (2008)

*Assume 1 ppm = 1 $mg.L^{-1}$ (Chiou *et al*., 1977).

Table 9.6 The effects of sodium carbonate and bicarbonate treatments in citrus fruit

Description of SC or SB Solution	Exposure Time	Solution Temperature	Fruit	Effect	Reference
4% or 6% SC	120 seconds	40.6°C or 43.3°C	Oranges	Significant reduction in green mould	Smilanick *et al.* (1997)
3% SC	60 seconds	56°C or 61°C	Navel oranges	Rind injury	Smilanick *et al.* (1999)
3% SC	150 seconds	50°C	Clementine mandarins	Significant reduction in blue and green mould, no visible injury to the fruit	Palou *et al.* (2002)
2% or 3% SB	60 or 150 seconds	Room temperature (20±1°C)		Reduced incidence of blue and green mould by 40-60%	
2% SB	-	-	Grapefruit	Reduced decay as a result of green mould by 61%	Porat *et al.* (2002)
5% SC	-	-	Fairchild mandarin	Resulted in accumulation of scoparone, associated with a reduction in decay	Venditti *et al.* (2005)
			Biondo comune oranges	Green mould decay reduced by 97.2% and blue mould decay reduced by 93.9%	

'-' Information not provided in the research article.
SB, sodium bicarbonate; SC, sodium carbonate.

Table 9.7 The effects of different postharvest biocontrol agents used on citrus fruit

Type of Biocontrol Agent	Fruit	Effect	Reference
Candida famata isolated from fig leaves	Orange	95-100% reduction in infected fruit in terms of green mould	Arras (1996)
		Promoted the production of scoparone	
Candida fermentati isolated from tomato fruit surface	Grapefruit	Production of fungal cell wall degrading enzymes resulting in a reduction in green mould infected fruit	Bar-Shimon *et al.* (2004)
		Reduced infected wounds to 10% in yeast-treated wounds, compared to 100%	
Yeast isolates (B13 and Grape)	Navel oranges and lemons	Prevented the onset of decay as a result of green mould	Abraham *et al.* (2010)
		Suitable as a preventative mode of action rather than curative	
Pichia guilliermondii (Z1)	Valencia-late oranges	Significant reduction in blue mould by at least 85%, independent of temperature or relative humidity	Lahlali *et al.* (2011)
		Well suited as a preventative mode of action	

Table 9.8 The effects of combined pre-packaging treatments applied to citrus fruit

Number	Description of Treatments	Additional Information	Fruit	Effect	Reference
1	Hot water	43 or 46°C for 180 seconds	Valencia oranges	Significant reduction in citrus black spot lesions	Korf *et al*. (2001)
	Chlorine	100 µm.mL^{-1} and 15 µg.mL^{-1}			
	High pressure spray	20-35 kPa			
	Polyethylene wax	-			
2	Biocontrol	*Bacillus* F1	Valencia and Shamouti oranges	Significant reduction in both blue and green mould.	Obagwu and Korsten (2003)
	Hot water	45°C for 120 seconds			
3	Biocontrol	*Bacillus* F1	Valencia and Shamouti oranges	Significant reduction in both blue and green mould.	Obagwu and Korsten (2003)
	SB	1% Solution			
4	Thermal curing	35-36°C for 72 hours	Nagami kumquat	Reduction in fruit decay	Ben-Yehoshua *et al*. (2005)
	UV-C Irradiation	0.5, 1.5, or 3.0 kJ^{-2}			
5	Hot water dipping	52°C for 120 seconds	Washington Navel orange	Reduction in fruit decay	Ben-Yehoshua *et al*. (2005)
	UV-C Irradiation	0.5, 1.5, or 3.0 kJ^{-2}			
6	SB	1% Solution	Eureka lemons	Incidence of green mould reduced to 22%	Smilanick *et al*. (2005)
	Imazalil	10 µg.mL^{-1}			
7	Free chlorine	100 µg.mL^{-1}	Satsuma mandarin	Closing of stomatal cracks by melting epicuticilar wax, reduction in decay caused by blue and green mould, Reduced weight loss	Sen *et al*. (2007)
	Hot water dipping	53°C for 180 seconds			
8	Biocontrol	*Bacillus amyloliquefaciens* HF-01	Wuzishatang ju mandarin	Firmer fruit, high ascobic acid, reduced levels of TSS, weight loss and decay	Hong *et al*. (2014)
	Hot water	45°C for 120 seconds			
	SB	1% or 2% Solution			

10. APPENDIX B – INTEGRATED POSTHARVEST CITRUS TREATMENT UNIT

Table 10.1 Experimental design for Chapter 6

	Treatment Combination			
Treatment	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**
1	Anolyte water - dipping time of 30 seconds	Hot water - temperature of 53°C	Dip time of 20 seconds	With B13
2				No B13
3			Dip time of 30 seconds	With B13
4				No B13
5		Hot water - temperature of 60°C	Dip time of 20 seconds	With B13
6				No B13
7			Dip time of 30 seconds	With B13
8				No B13
9		Tap water	Dip time of 20 seconds	With B13
10				No B13
11			Dip time of 30 seconds	With B13
12				No B13
13	Anolyte water - dipping time of 60 seconds	Hot water - temperature of 53°C	Dip time of 20 seconds	With B13
14				No B13
15			Dip time of 30 seconds	With B13
16				No B13
17		Hot water - temperature of 60°C	Dip time of 20 seconds	With B13
18				No B13
19			Dip time of 30 seconds	With B13
20				No B13
21		Tap water	Dip time of 20 seconds	With B13
22				No B13
23			Dip time of 30 seconds	With B13
24				No B13
25	Tap water	Hot water - temperature of 53°C	Dip time of 20 seconds	With B13
26				No B13
27			Dip time of 30 seconds	With B13
28				No B13
29		Hot water - temperature of 60°C	Dip time of 20 seconds	With B13
30				No B13
31			Dip time of 30 seconds	With B13
32				No B13
33		Tap water	Dip time of 20 seconds	With B13
34				No B13
35			Dip time of 30 seconds	With B13
36				No B13

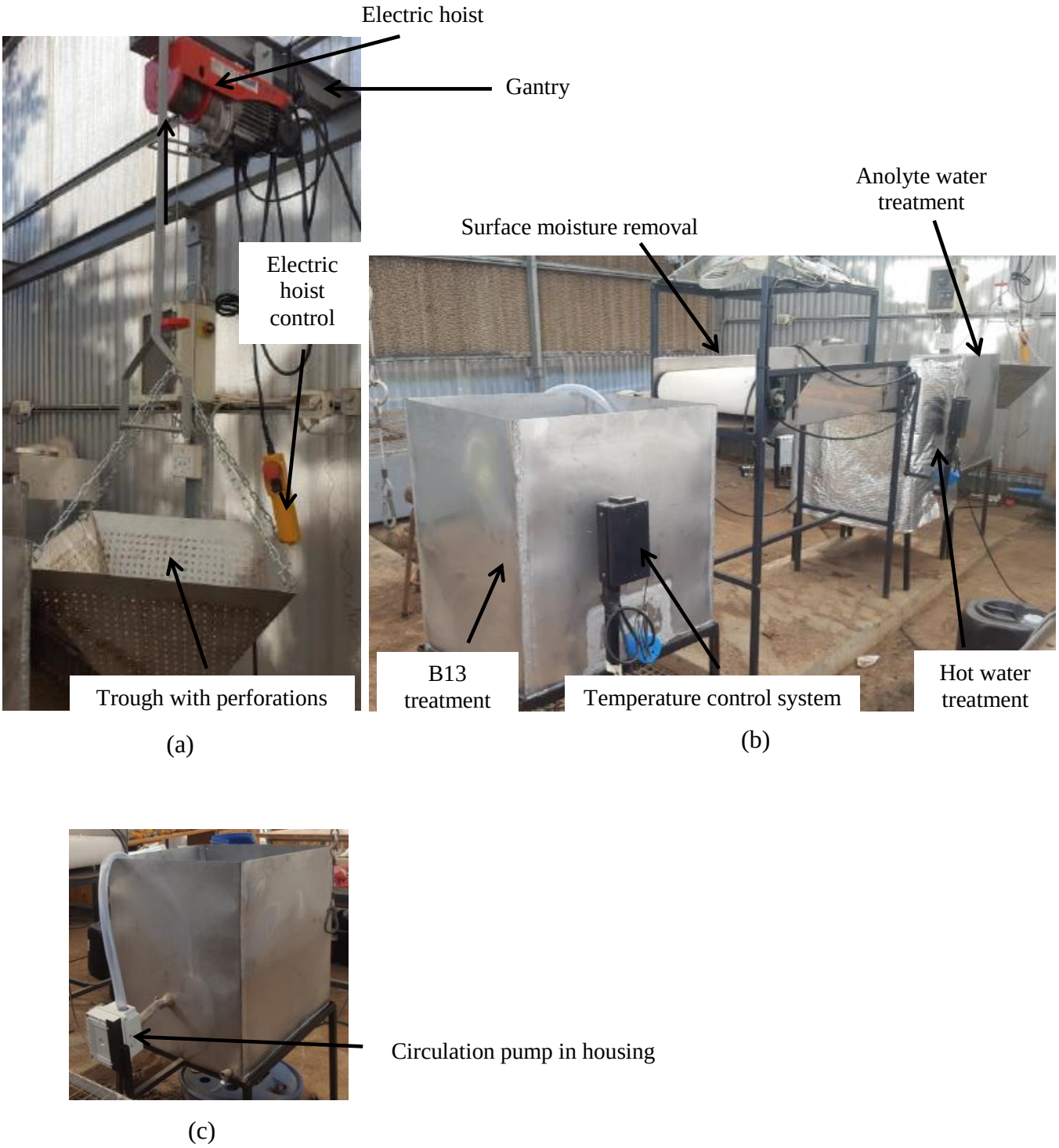

Figure 10.1 Components of the IPCTU (a) perforated trough, (b) complete unit and (c) circulation pump