The effects of different pre-packaging treatments on the quality of kumquat fruit

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ABSTRACT
This study aimed at determining the combined effect of pre-packaging treatments. Two surface disinfection (chlorinated and anolyte water), hot water and biocontrol treatments were used. The decay incidence, physiological weight loss (PWL), peel colour, firmness, moisture content (MC) and total soluble solids (TSS) were investigated on kumquat infected with green and blue mould. Fruit subjected to combined treatments displayed better quality, compared to individual treatments. Fruit treated with an integrated treatment of anolyte water, hot water and biocontrol displayed no visible mould growth, the least variation in peel firmness and the least reduction in MC during the 28-day storage period. Chlorinated water only produced the highest decay (13.62%) while fruit subjected to anolyte water only displayed the lowest decay (4.08%) by Day 28. Based on the results, it is recommended that the integration of anolyte water, hot water and biocontrol were among the most effective treatments in maintaining fruit quality.

Introduction
The kumquat (genus *Fortunella*), as with other citrus fruit is classified as non-climacteric (Ladaniya, 2008). However, the postharvest shelf life of kumquat fruit is relatively short due to the effect of *Penicillium*, which result in high levels of decay (Schirra, Angioni, Cabras, D’Aquino, & Palma, 2011). *Penicillium* has been identified as the leading pathological cause of postharvest decay in citrus fruit (Ladaniya, 2008; Youssef, Sanzani, Ligorio, Ippolito, & Terry, 2014). This necessitates the need for further research to be conducted to mitigate decay and loss caused by this prevalent fungal infection. Few studies have focussed on kumquat fruit, compared to orange, grapefruit, soft citrus and lemon varieties. There exists a large market for kumquat fruit particularly in the European Union and United Kingdom being the major export destinations from South Africa. 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 [DAFF], 2014).

Extensive research on individual pre-packaging treatments such as hot water treatments, surface disinfection and biocontrol agents on citrus have been documented to alleviate decay caused by *Penicillium*. However, research on integrating these treatments has created interest due to the potential to improve quality and reduce decay. There is an increasing need to search for alternate and more environmentally friendly treatments to address the issue of fruit decay and minimise losses. Hot water treatments have been found to improve the shelf life and quality of kumquat fruit with the accumulation of scoparone in the flavedo (Ben-Yehoshua, Rodov, D’hallewin, & Dore, 2005; Schirra et al., 2011). A study by Hall (1986) investigated the use of integrated postharvest treatments of chlorine, sodium o-phenylphenate, thiabendazole, 2,4-dichlorophenoxyacetic acid and waxes on kumquat fruit, which improved fruit quality, compared to individual
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Biocontrol: commercially available B13 (strain of Anolyte or electrochemically activated water disinfection) was conducted to determine which is a more effective disinfectant. These findings will then be applied to design a postharvest treatment unit in subsequent research.

Materials and methods

Sample fruit production

Nagami (Fortunella margarita) is the most important kumquat cultivar exported from South Africa. Kumquat samples were obtained from Rooister Boerdery in the Letsitele region, just outside Tzaneen, 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. The climate is hot and dry and the trees require regular irrigation. Dicarzol® and white sugar as well as Abamec® and oil are sprayed onto the trees to combat thrips. Fruit are harvested by hand using clippers, the timing based on their peel colour (yellow to orange). Commercially mature kumquat fruits were transported via truck to the Pietermaritzburg Fresh Fruit Market in Mkondeni, approximately 3 km from the laboratories. This was to ensure minimal fruit exposure to temperature fluctuations between harvesting and sampling. A total of 1100 fruit (approximately 12 kg) were used for this laboratory study.

Pre-packaging disinfection treatments

The preliminary experiment was conducted under laboratory conditions at the Food Science and Agricultural Engineering Laboratory at UKZN. The main pre-packaging treatments for this experiment were: (1) chlorinated water and (2) anolyte water as the disinfectant treatments; (3) hot water as the curative treatment; (4) B13 biocontrol (a strain of the yeast Candida fermentati) as the preventative treatment and (5) a control treatment. These treatments were selected as they encompass disinfectant, curative and preventative methods, respectively. This experiment was to determine the relative efficacy of chlorinated water or anolyte water as the disinfectant treatment in the design of an integrated pre-packaging treatment unit. The fruits were subjected to 12 treatments as follows: (1) chlorinated water; (2) anolyte water; (3) hot water; (4) biocontrol; (5) combined chlorine and hot water; (6) combined chlorine and biocontrol; (7) combined chlorine, hot water and biocontrol; (8) combined anolyte and hot water; (9) combined anolyte and biocontrol; (10) combined anolyte, hot water and biocontrol; (11) hot water and biocontrol and (12) control. The following procedures were adopted for the pre-treatment of the kumquat fruit:

- Chlorinated water: a concentration of 100 mg/kg was used at a pH of 7.0–7.2 (Beuchat & Ryu, 1997; Suslow, 1997; M.D. Laing, personal communication, 23 June 2014). This was achieved by adding 0.734 g of sodium hypochlorite (NaClO) granules to 5 litres of water (as per the manufacturer’s specifications). The fruits were immersed in the chlorinated water for 30 seconds. The temperature of the water was measured to be 22°C. The concentration and pH of the chlorinated water was monitored using the Hydron micro chlorine tester and the Hydron pH and sanitiser test kit, respectively. This ensured that the correct concentration and pH was attained. Upon removal the fruits were air-dried.
- Anolyte or electrochemical activated water disinfection: 5 litres of commercially available anolyte water was used with a dipping time of 30 seconds at a pH of 6–7 at a temperature of 22°C (Lesar, 2002; A. Louw, personal communication, 1 September, 2014). The concentration and pH of the anolyte water was monitored using the Hydron micro chlorine tester and the Hydron pH and sanitiser test kit, respectively. Upon removal the fruits were air-dried.
- 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 fruits were then immersed in heated water for 20 seconds (M.D. Laing, personal communication, 23 June 2014; Schirra et al., 2011). Once removed the fruits were air-dried.
- B13 Biocontrol: commercially available B13 (strain of Candida fermentati) yeast formulated by Plant Health Products (Pty) Ltd located in Nottingham Road, South Africa was used. The recommended commercial concentration was 100 g per 100 litres of warm water (I. Basdew, personal communication, 4 September 2014). 10 g of the B13 yeast 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 fruits were immersed for 60 seconds and air-dried upon removal.
- Control: fruits were dipped in potable water for 10 seconds at 23.5°C. Upon removal the fruits were air-dried.

Once the treatments were applied, the fruits were stored in their respective batches in the Food Science and Agricultural Engineering Laboratory at ambient conditions for 28 days. Three HOBO® data loggers were used to measure the ambient conditions (temperature and relative humidity) of the storage area. Once the storage period had concluded, the BoxCar® Pro 4.3 software was used to retrieve the temperature and relative humidity data from the data loggers for analysis.
**Experimental design**

A randomised complete block design (RCBD) was used as the experimental design as it accounts for any variations in the samples (Compton, 1994). The experiment was performed in triplicate with three replications. This was conducted on kumquat fruit inoculated with *Penicillium digitatum* (green mould) and separately for kumquats inoculated with *Penicillium italicum* (blue mould). Fruits were inoculated prior to the application of the treatments.

Two surface disinfecting treatments of chlorinated water (A) and analyte water (B) were used. One curative treatment of hot water (C) and one preventative treatment of a biocontrol (D) was used. These treatments were applied individually and in combination of A, B, C, D, AC, AD, ACD, BC, BD, BCD, CD and a control of tap water. After treatment the fruits were stored at ambient conditions of 23°C and 54% relative humidity for 28 days. Fruits 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.

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

All laboratory utensils and apparatus were sterilised for 15 minutes at 121°C using a vertical-type steam steriliser. About 10 mL of potato dextrose agar (35 g.L⁻¹ water) were added to petri dishes and allowed to solidify for one hour. The plates were then used to culture *Penicillium digitatum* and *Penicillium italicum*, which were isolated from infected oranges. The petri dishes were incubated for 3–5 days at 28°C to promote hyphae development. Once hyphae development was complete, a ‘clean’ uncontaminated portion (5 mm × 5 mm) of the mould was sub-cultured from the initial isolation to new potato dextrose agar petri dishes. Seven plates were used to culture *Penicillium digitatum* and seven plates for *Penicillium italicum*. These plates were then incubated for a further 7–14 days at 28°C for fungi sporulation. *Penicillium italicum* was observed to take a longer period to develop, compared to the *Penicillium digitatum* and as a result a further 7 days were allocated for sporulation. Once the sporulation was complete, the conidia were harvested by adding approximately 20 mL of sterile distilled water to each of the petri dishes (Smilanick et al., 1999). The conidia were then loosened with the aid of a laboratory hockey stick. The conidia suspensions were collected in two sterilised glass jars for each mould.

**Sample preparation**

Untreated kumquat fruits were selected, based on uniformity of size, colour and damage (Hong, Lee, & Kim, 2007). Fruit that showed signs of damage or deformity were discarded. The fruits 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 fruits were dried using laboratory paper towels. The fruits were sorted into 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 the green mould and the remaining 36 batches were inoculated with blue mould.

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

*Penicillium digitatum* and *Penicillium italicum* conidia that had been prepared, as previously discussed, were used. Conidia suspension concentrations were quantified using a Neubauer hemocytometer and then diluted to the desired concentration using sterilised distilled 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, Laing, & Bower, 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 conidia suspension of *Penicillium digitatum* at a concentration of 1 × 10⁶ conidia.mL⁻¹ (Abraham et al., 2010). The same procedure was followed for inoculating the remaining fruit with the *Penicillium italicum* conidia suspension. After a further 24 hours the 12 pre-treatments were applied to the fruit, which were then stored at ambient conditions (23°C and 54% relative humidity).

**Data collection and analysis**

The effect of the treatments on the kumquat fruits were evaluated based on the change in the physical, chemical and microbiological quality of the fruit. The physical quality parameters that were investigated included the physiological weight loss (PWL), peel firmness and peel colour. The chemical quality parameters that were investigated, included the moisture content (MC) and total soluble solids (TSS) and the microbiological quality parameter was based on the decay incidence as a result of *Penicillium digitatum* and *Penicillium italicum*.

**Decay incidence**

Decay incidence 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 Vernier callipers (0.05 mm precision). In addition, the number of fruit that had developed fungal growth per batch was calculated and expressed as a percent on each sampling interval (Abraham et al., 2010; Hong et al., 2007; Schirra et al., 2011).

**Physiological weight loss**

Kumquat fruits were individually weighed using a Mettler PJ 300 scale 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) (Hong et al., 2007; Singh & Reddy, 2006).

**Peel colour**

The peel colour was measured using a Konica Minolta CR-400 colorimeter. 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 obtained. The parameters L*, a* and b* were measured (Li,
Zhong, Peng, Li, & Zheng, 2008). The hue angle could then be calculated using the methods by Choi, Kim, and Lee (2002).

**Peel firmness**

The Instron Universal Testing Machine (Model 3345) 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 fruits 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, Martinez-Romero, Serrano, & Riquelme, 1998).

**Peel moisture content**

Each fruit was cut in half with the pulp removed from one half. Approximately 2 g of the peel was placed on to a piece of aluminum foil. The weight of the foil and peel were measured using a Mettler PJ 300 scale. The samples were then placed in a hot air oven at 105°C for 24 hours (Jaliliantabar, Lorestani, & Gholami, 2013). After drying for 24 hours, the samples were then reweighed. The peel MC was calculated on a wet basis (Singh & Reddy, 2006).

**Total soluble solids**

The TSS 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) (Schirra et al., 2011; Valero et al., 1998). The prism was cleaned with 99.9% ethanol and then with distilled water, using a soft cloth between samples.

**Statistical data analysis**

The statistical analysis was performed using the GenStat software, 14th 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; Duncan, 1955; Workneh et al., 2011).

**Results and discussion**

**Decay incidence**

Table 1 presents the decay incidence on the surface of kumquat fruit as a result of green mould. The treatment and storage period were found to have a highly significant (P ≤ 0.001) influence on the decay incidence of kumquat fruit. No visible mould growth was observed between Day 0 and Day 7 for all treatments. On Day 14, a notable increase in the mould formation was measured at 4.48% of the surface area for chlorine only. At Day 28 the mould formation had grown substantially, amounting to 13.62%. In addition to the chlorine treatment having the greatest decay incidence, a total of 66% of the fruit in this batch showed visible signs of mould development by Day 28 (Table 2). This was followed by the biocontrol treatment with 44% of fruit displaying visible decay (Day 28). This corresponds to the findings by Abraham et al. (2010) in which the discrete use of the yeast biocontrol was better as a preventative treatment than as a curative treatment. The combination treatment of anolyte water, hot water and the biocontrol 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 blue and green mould of oranges due to the combination of hot water (45°C for 120 seconds) and biocontrol (Bacillus F1). The lack of mould formation in the control samples could be attributed to the environmental conditions in which the fruits were stored. This may have not been conducive for mould formation. The mould could have developed below

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**Table 1.** The development of green mould (Penicillium digitatum) on the surface of the kumquat fruit (%) subjected to different pre-packaging treatments.

| Treatment                      | 0       | 7       | 14      | 21      | 28      |
|-------------------------------|---------|---------|---------|---------|---------|
| Chlorine water                | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 4.48\(\pm1.3\) | 8.41\(\pm0.8\) | 13.62\(\pm0.8\) |
| Anolyte water                 | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) |
| Hot water (HW)                | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) |
| Biocontrol                    | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 1.05\(\pm0.1\) | 1.05\(\pm0.1\) | 3.01\(\pm0.8\) |
| Chlorine + HW                 | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) |
| Chlorine + biocontrol         | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) |
| Chlorine + HW + biocontrol    | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.52\(\pm0.9\) | 2.62\(\pm0.6\) |
| Anolyte + HW                  | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 1.81\(\pm0.7\) | 1.81\(\pm0.7\) | 1.81\(\pm0.7\) |
| Anolyte + biocontrol          | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.22\(\pm0.4\) | 3.50\(\pm1.2\) | 4.26\(\pm1.5\) |
| Anolyte + HW + biocontrol     | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) |
| HW + biocontrol               | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) |
| Control                       | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) | 0.00\(\pm0.0\) |

**Significance**

| Treatment (A) | ** | ** |
|---------------|---|---|
| Storage period (B) | ** | x |
| AB            |   |   |

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).
the surface of the fruit and not yet visible on the fruit surface. The anolyte and hot water treatment resulted in a mould formation of 1.81% on Day 14, which remained constant for the remaining storage period. Similarly chlorination and hot water had a constant decay incidence of 4.71%. Hot water only and the combination of chlorination and biocontrol showed favourable results with low decay incidences of 1.31% and 1.36%, respectively, by Day 28.

The two-way interaction between the treatment and storage period had a significant influence on the decay incidence as a result of green mould at \( P \leq 0.05 \). As time progressed the amount of decay caused by green mould increased as it is a highly prolific fungi (Hong et al., 2007; Schirra et al., 2011). Hong 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. More significantly the reduction in decay is 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 have also resulted in a reduction in the epiphytic micro-organism population, which has proved to be beneficial (Hong 2007). As found by Abraham et al. (2010) the biocontrol yeast B13 is better suited as a preventative treatment. 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). Therefore, the addition of chlorine or anolyte water as a disinfectant to remove some of the existing surface pathogens resulted in a lower decay incidence. Furthermore, with the action of the hot water treatment to induce fruit resistance as in the case of anolyte, hot water and biocontrol, no incidence of decay was observed. Kim, Ben-Yehoshua, Shapiro, Henis, and Carmeli (1991) observed an increase in scoparone in citrus fruit after inoculation with Penicillium 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 that the presence of pathogens elicited fruit resistance. Lesar (2002) found that a dilution of anolyte water of 1:5 and 1:10 resulted in 100% spore eradication in citrus fruit with an exposure time ranging from 30 to 300 seconds.

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 and the biocontrol and (2) hot water and the biocontrol were most beneficial in preventing green mould decay in kumquat fruit.

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 blue mould-infected fruit have subsequently been omitted from the discussion of results.

### Physiological weight loss

The treatment and storage period were found to have a highly significant \( (P \leq 0.001) \) effect on the PWL of kumquat fruit as indicated in Table 3. The four single treatments of (1) chlorinated water; (2) anolyte water; (3) hot water and (4) biocontrol 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%. A large increase in the PWL can be observed between Days 14 and 21 and 28, particularly in the combined treatment of chloride, hot water and biocontrol.

The two-way interaction between the treatments and the storage period was also found to be highly significant \( (P \leq 0.001) \) 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). The combined treatments proved to be better at reducing the PWL of kumquat fruit, compared to individual

### Table 2: Percentage of decayed fruit due to green mould (Penicillium digitatum).

| Treatment                  | 0   | 7   | 14  | 21  | 28  | *Total % of decayed fruit |
|----------------------------|-----|-----|-----|-----|-----|--------------------------|
| Chlorine water             | 0   | 0   | 22  | 11  | 33  | 66                       |
| Anolyte water              | 0   | 0   | 0   | 11  | 22  | 33                       |
| Hot water (HW)             | 0   | 0   | 0   | 11  | 11  | 22                       |
| Biocontrol                 | 0   | 22  | 11  | 11  | 11  | 44                       |
| Chlorine + HW              | 0   | 0   | 0   | 11  | 11  | 22                       |
| Chlorine + biocontrol      | 0   | 0   | 0   | 11  | 11  | 22                       |
| Chlorine + HW + biocontrol | 0   | 0   | 0   | 11  | 11  | 22                       |
| Anolyte + HW               | 0   | 0   | 11  | 11  | 11  | 33                       |
| Anolyte + biocontrol       | 0   | 11  | 11  | 11  | 11  | 33                       |
| Anolyte + HW + biocontrol  | 0   | 0   | 0   | 0   | 0   | 0                        |
| HW + biocontrol            | 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.

*Porcentaje total de fruto deteriorado al final del día 28 del periodo de almacenamiento.
treatments. Treatments incorporating anolyte water reduced the PWL to a greater extent, compared to the same treatments using chlorinated water instead.

**Peel colour**

The changes in the hue angle of the kumquat fruit is presented in Table 4. 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. Smilanick, Mansour, and Sorensen (2006) also did not find a significant difference in the hue angle of treated and untreated citrus fruit. This is indicative of a colour change from a yellow-lime to an orange-yellow. Chlorine treatment and control samples displayed 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) chlorine and hot water and (4) chlorine and biocontrol were not significantly different. Similarly (1) biocontrol, (2) chlorine, hot water and biocontrol, (3) anolyte and hot water, (4) anolyte and biocontrol and (5) anolyte, hot water and biocontrol treatments were not significantly different. 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. A decrease in the hue angle can be indicative of ripening. Therefore the treatments which show the least decrease in the hue angle are the combined treatments, compared to individual treatments.

**Table 3.** The physiological weight loss (%) of green mould-inoculated (*Penicillium digitatum*) kumquat fruit subjected to different pre-packaging treatments.

**Table 3.** La pérdida de peso psicológica (%) del kumquat con moho verde inoculado (*Penicillium digitatum*) sujeto a diferentes tratamientos de preensados.

**Table 4.** The hue angle (degrees) of green mould-inoculated (*Penicillium digitatum*) kumquat fruit subjected to different pre-packaging treatments.

**Tabla 4.** El ángulo de tonalidad cromática (grados) del kumquat con moho verde inoculado (*Penicillium digitatum*) sujeto a diferentes tratamientos de preensados.
Peel firmness

Table 5 presents the change in firmness of 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 Day 14 (7.30 N) and Day 28 (9.04 N). However, chlorine-treated kumquats displayed the least firmness (7.05 N), which is concomitant with the greatest PWL (86.17%) and lowest MC (40.53%) as indicated in Tables 3 and 6, respectively. Chlorination and biocontrol caused a low firmness on Day 28 of 7.10 N.

The firmness in citrus fruit depends primarily on turgidity and weight loss (Hong et al., 2007; Olmo, Nadas, & García, 2000). Olmo et al. (2000) found that a decrease in the firmness coincided with an increase in the weight loss. This can account for the substantial reduction in the firmness of chlorine-treated kumquats inoculated with green mould, which is related to the excessive loss in moisture and weight loss producing a tough and shriveled exterior. Studies by Rodov, Agar, Peretz, Nafussi, Kim, & Ben-Yehoshua (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 MC resulting in a drying effect and softening of the peel tissue. Ladaniya (2008) found that with increasing moisture loss, the peel of citrus fruit becomes tough and leathery, resulting in higher puncture resistance. This could account for the increase in firmness particularly between Days 21 and 28 in control fruit (9.04 N). The post-harvest storage of fruit is associated with 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 firm fruit tissue. The combined treatment of a biocontrol agent (Bacillus amyloliquefaciens HFO1), 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 that the combination of hot water and chlorine to be effective in extending the shelf-life of citrus fruit (Sen, Knay, Karacal, & Berolini, 2007). However, the addition of a biocontrol agent further improves the efficacy (Sen et al., 2007). This study found that the use of anolyte water as a disinfectant in integrated treatments was more effective in maintaining the fruit firmness compared to chlorine. The combined treatments using anolyte water as the disinfectant maintained fruit firmness better than treatments in which chlorine had been the disinfectant. In addition, the treatment of hot water and biocontrol produced similar beneficial results.

Moisture content

Table 6 indicates the changes in the MC as a result of different pre-packaging treatments of kumquat fruit inoculated with green mould (Penicillium digitatum). Many studies have found that the combination of hot water and chlorine to be effective in extending the shelf-life of citrus fruit (Sen, Knay, Karacal, & Berolini, 2007). However, the addition of a biocontrol agent further improves the efficacy (Sen et al., 2007). This study found that the use of anolyte water as a disinfectant in integrated treatments was more effective in maintaining the fruit firmness compared to chlorine. The combined treatments using anolyte water as the disinfectant maintained fruit firmness better than treatments in which chlorine had been the disinfectant. In addition, the treatment of hot water and biocontrol produced similar beneficial results.

### Table 5. Puncture force (N) of the kumquat fruit peel subjected to different pre-packaging treatments inoculated with green mould (Penicillium digitatum).

| Treatment                          | Storage period (days) |
|-----------------------------------|-----------------------|
|                                   | 0         | 7         | 14        | 21        | 28        |
| Chlorine water                    | 11.71±     | 6.99±(±0.7)| 6.10±(±0.8)| 6.99±(±0.4)| 7.05±(±2.3)|
| Anolyte water                     | 11.71±     | 10.60±(±1.3)| 8.08±(±0.4)| 7.05±(±0.7)| 7.56±(±0.9)|
| Hot water (HW)                    | 11.71±     | 7.82±(±0.3)| 7.57±(±0.2)| 7.04±(±1.9)| 7.90±(±1.5)|
| Biocontrol                        | 11.71±     | 7.38±(±0.6)| 7.41±(±0.2)| 8.30±(±0.2)| 8.48±(±0.5)|
| Chlorine + HW                     | 11.71±     | 7.63±(±0.6)| 7.98±(±0.8)| 7.84±(±0.4)| 7.20±(±0.3)|
| Chlorine + biocontrol             | 11.71±     | 7.66±(±0.0)| 7.90±(±0.6)| 7.06±(±1.5)| 7.10±(±0.3)|
| Chlorine + HW + biocontrol        | 11.71±     | 7.36±(±0.5)| 7.84±(±0.3)| 7.54±(±0.6)| 7.57±(±1.2)|
| Anolyte + HW                      | 11.71±     | 8.16±(±0.5)| 9.07±(±0.7)| 7.83±(±0.2)| 7.68±(±0.7)|
| Anolyte + biocontrol              | 11.71±     | 7.98±(±0.5)| 8.90±(±0.5)| 5.86±(±0.4)| 7.57±(±0.2)|
| Anolyte + HW + biocontrol         | 11.71±     | 8.71±(±1.3)| 7.98±(±0.8)| 8.51±(±0.4)| 7.85±(±0.6)|
| HW + biocontrol                   | 11.71±     | 7.34±(±0.6)| 7.88±(±0.4)| 7.55±(±0.8)| 7.82±(±0.5)|
| Control                           | 11.71±     | 8.12±(±0.3)| 7.30±(±1.1)| 8.75±(±0.8)| 9.04±(±0.4)|

Significance

- **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 ($P \leq 0.05$) ($n = 3$).
- **No significativo o significativo a $P \leq 0.05$ o $P \leq 0.001$, respectivamente. Los promedios en una misma columna seguidos de la misma letra/s no son significativamente distintos entre ellos según el Test de rango múltiple de Duncan ($P \leq 0.05$) ($n = 3$).
Table 6. Contenido de humedad en la piel (% peso mojado) del kumquat con moho verde inoculado (Penicillium digitatum) sujeto a diferentes tratamientos de preenvasado.

| Treatment             | Storage period (days) | 0  | 7  | 14 | 21 | 28 |
|-----------------------|-----------------------|----|----|----|----|----|
| Chlorine water        |                       | 62.8 (±0.0) | 71.9 (±0.1) | 64.2 (±0.0) | 56.9 (±0.0) | 40.3 (±0.1) |
| Anolyte water         |                       | 62.8 (±0.0) | 74.0 (±0.0) | 63.6 (±0.0) | 60.0 (±0.0) | 50.7 (±0.0) |
| Hot water (HW)        |                       | 62.8 (±0.0) | 66.0 (±0.1) | 66.8 (±0.0) | 59.1 (±0.0) | 50.1 (±0.0) |
| Biocontrol            |                       | 62.8 (±0.0) | 72.6 (±0.0) | 60.7 (±0.0) | 59.5 (±0.1) | 48.0 (±0.1) |
| Chlorine + HW         |                       | 62.8 (±0.0) | 70.3 (±0.0) | 67.7 (±0.0) | 59.1 (±0.0) | 58.8 (±0.1) |
| Chlorine + biocontrol |                       | 62.8 (±0.0) | 70.5 (±0.0) | 66.8 (±0.0) | 58.2 (±0.0) | 50.3 (±0.0) |
| Chlorine + HW + biocontrol |               | 62.8 (±0.0) | 68.5 (±0.0) | 67.1 (±0.0) | 61.2 (±0.0) | 51.1 (±0.1) |
| Anolyte + HW          |                       | 62.8 (±0.0) | 74.8 (±0.0) | 64.6 (±0.0) | 63.9 (±0.0) | 56.6 (±0.0) |
| Anolyte + biocontrol  |                       | 62.8 (±0.0) | 70.4 (±0.0) | 63.1 (±0.0) | 58.4 (±0.0) | 52.6 (±0.0) |
| Anolyte + HW + biocontrol |                 | 62.8 (±0.0) | 66.0 (±0.0) | 67.0 (±0.0) | 62.9 (±0.0) | 57.0 (±0.0) |
| HW + biocontrol       |                       | 62.8 (±0.0) | 71.2 (±0.0) | 65.1 (±0.0) | 60.9 (±0.0) | 51.7 (±0.0) |
| Control               |                       | 62.8 (±0.0) | 68.3 (±0.0) | 64.0 (±0.0) | 56.0 (±0.0) | 42.3 (±0.0) |

Significance
Treatment (A) **
Storage period (B) **
AB **

NS, *, ** no-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).

Citrus fruit have a high MC in both the pulp and peel (Chien, Sheu, & Lin, 2007; Ghanema, Mihoubib, Kechaoua, & Mihoubic, 2012). Once harvested the fruit loses excessive moisture from the peel via transpiration and respiration, promoting the onset of decay and reduced shelf-life (Purvis, 1983). The combined treatments of (1) anolyte water, hot water and biocontrol and (2) chlorine and hot water were effective in hindering the loss in moisture, therefore producing fruit of higher MC.

Total soluble solids
The changes in the TSS of kumquat fruit inoculated with green mould subjected to different pre-packaging treatments are presented in Table 7. 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 the control samples also caused 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, chlorine 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 Day 14 to Day 28. The TSS of kumquats in individual treatments and the control were higher, compared to those exposed to integrated pre-packaging treatments.

An increase in the TSS of citrus fruit have been observed by D’holewin, Arras, Castia, and Piga (1994), Ladaniya (2008), Olmo et al. (2000) and Rodov et al. (2000) which can be attributed to a loss in water after harvest. Therefore, as the fruit matures an increase in the TSS is expected. However;

Table 7. Total soluble solids (°Brix) of green mould-inoculated (Penicillium digitatum) kumquat fruit subjected to different pre-packaging treatments.

| Treatment               | Storage period (days) | 0  | 7  | 14 | 21 | 28 |
|-------------------------|-----------------------|----|----|----|----|----|
| Chlorine water          |                       | 10.2 (±0.0) | 12.0 (±0.2) | 14.9 (±0.2) | 16.0 (±0.7) | 18.5 (±0.7) |
| Anolyte water           |                       | 10.2 (±0.0) | 12.4 (±1.4) | 14.3 (±0.6) | 15.3 (±0.9) | 16.3 (±0.6) |
| Hot water (HW)          |                       | 10.2 (±0.0) | 13.4 (±1.5) | 14.0 (±1.0) | 15.6 (±0.1) | 16.3 (±0.6) |
| Biocontrol              |                       | 10.2 (±0.0) | 12.0 (±0.8) | 15.6 (±1.3) | 15.9 (±1.5) | 16.4 (±1.2) |
| Chlorine + HW           |                       | 10.2 (±0.0) | 13.5 (±1.3) | 14.5 (±1.4) | 16.8 (±2.2) | 15.8 (±0.7) |
| Chlorine + biocontrol   |                       | 10.2 (±0.0) | 12.2 (±0.5) | 13.5 (±0.7) | 16.4 (±0.9) | 16.0 (±1.8) |
| Chlorine + HW + biocontrol |                  | 10.2 (±0.0) | 12.9 (±1.2) | 13.4 (±0.8) | 16.5 (±2.0) | 16.7 (±1.0) |
| Anolyte + HW            |                       | 10.2 (±0.0) | 12.1 (±0.4) | 15.1 (±1.5) | 16.0 (±2.2) | 16.0 (±1.1) |
| Anolyte + biocontrol    |                       | 10.2 (±0.0) | 10.8 (±0.4) | 14.3 (±0.7) | 16.1 (±2.3) | 15.8 (±1.9) |
| Anolyte + HW + biocontrol |                 | 10.2 (±0.0) | 9.8 (±0.3)  | 14.2 (±0.4) | 15.2 (±0.4) | 15.8 (±0.7) |
| HW + biocontrol         |                       | 10.2 (±0.0) | 11.6 (±0.9) | 13.1 (±0.5) | 15.2 (±0.4) | 16.6 (±0.3) |
| Control                 |                       | 10.2 (±0.0) | 11.9 (±0.6) | 15.6 (±0.9) | 16.1 (±0.9) | 17.8 (±0.9) |

Significance
Treatment (A) NS
Storage period (B) NS
AB NS

NS, *, ** no-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).
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 (Roongruangsi, Rattanapanone, Lekswasdi, & Boonyakiat, 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 was lower, compared to the 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 may be beneficial in reducing the rate of increase of the TSS, which is an indication of a slower maturation rate. The use of (1) anolyte water, hot water and biocontrol and (2) chlorine and hot water were found to be the most effective treatments in reducing the rate of maturation of kumquat fruit in terms of the TSS.

Conclusion

This study investigated the effects of chlorinated water, anolyte water, hot water and a biocontrol agent (strain of Candida fermentati strain B13) pre-packaging treatments on the quality of kumquat fruit. The study revealed that integrated pre-packaging treatments were more effective in reducing the onset of decay as a result of green mould, compared to single treatments on kumquat fruit. The application of anolyte water as a disinfectant caused better results in terms of decay incidence, PWL, firmness, MC and TSS than chlorinated water. Anolyte water combined with hot water resulted in firmer fruit with high MC. 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 incidence, 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 incidence, 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 are effective in maintaining desirable fruit quality. The practical implications of this study is that it can be adapted on a commercial scale to treat kumquat fruit without the use of synthetic fungicides after harvest but rather using more environmentally friendly treatments.

Disclosure statement

No potential conflict of interest was reported by the authors.

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