COMPARISON OF SAFE ALTERNATIVE DIPPING TREATMENTS TO MAINTAIN QUALITY OF ZUCCHINI

Mrs Willeke de Bruin, Mr Werner Rossouw and Prof Lise Korsten*

Institute for Food Nutrition and Well-Being and Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa

*Lise Korsten, Institute for Food Nutrition and Well-Being and Faculty of Natural and Agricultural Sciences, Department of Microbiology and Plant Pathology, Agricultural Building, Room 2-9, University of Pretoria, Pretoria, 0002, South Africa, e-mail of the corresponding author: lise.korsten@up.ac.za, Telephone number: +27 (0) 12 420 4097, Fax number: +27 (0) 12 420 4588

ABSTRACT

Decay and quality loss severely affect the marketability of fresh zucchini. Zucchinis are easily damaged during harvesting, - handling and - storage and no commercial treatment is currently available that can protect the fruit from desiccation, quality loss and decay. The aim of this study was to compare different environmentally friendly dipping treatments ($CaCl_2$, *Aloe vera*, warm water, chitosan, ascorbic- and citric acid) to retain quality of fresh zucchini. Treated fruit were evaluated for physiological, sensory and microbiological parameters after storage for seven and 14 days at 5.8°C and 85% relative humidity. The $CaCl_2$, *Aloe vera* and warm water treatments were the most effective in maintaining firmness and preventing an increase in the total microflora of the fruit. The outcome of this study shows that alternative control methods have potential for effective quality maintenance of fresh zucchini. Future studies should focus on alternative packaging materials in combination with these treatments.

PRACTICAL APPLICATIONS

Zucchinis are rich in carbohydrates, minerals and vitamins and a good nutritional additive to a balanced and healthy diet. Recent observations at retail level showed preventable quality losses, indicating the need to improve postharvest technologies for retaining the overall standard of the product after harvest and during the supply chain.

Quality parameters include firm texture, shiny, dark-green skin color, and freedom from shrivel and mechanical injuries. The assessment of different environmentally friendly dipping treatments indicated that $CaCl_2$, *Aloe vera* and warm water treatments could lead to improved quality maintenance of zucchinis. Practical applications stemming from these results include the ability to extend shelf-life and preserve the microbiological profile of these fruit after harvest. These treatments may serve as alternatives to chemical or synthetic treatments and may be considered as an inclusion to current postharvest management practices.

Keywords: Calcium chloride ($CaCl_2$), *Aloe vera*, warm water, chitosan, ascorbic acid, citric acid

1. Introduction

Zucchini (*Cucurbita pepo* L.) is an important vegetable crop worldwide (Esquinas-Alcazar and Gulick 1983) and one of the core food items commonly consumed by South Africans (Rautenbach and Venter 2010). In 2012, South Africa produced 186 813 tons of cucurbitaceous vegetables (FAOSTAT), while the average sales of zucchini in the Gauteng Province amounted to R3.1 million during 2009 (2011 personal communication Mr Mootane, South African Department of Agriculture, unreferenced). Zucchinis are a rich source of natural antioxidants such as beta-carotene, folic acid, vitamins C and E (Kami *et al.* 2011), making it a valuable nutritional vegetable for consumers.

Quality loss, decay and general deterioration of zucchini are a major concern for growers from production, during harvesting, packing, transporting, storage and after sales (Kader 1983). Since the fruit is harvested physiologically immature (Avena-Bustillos and Krochta 1994), it still has a poorly developed cuticle that is easily damaged during harvesting and subsequent handling. Fruit skin injuries can lead to abnormal cellular growth and microbial decay (Avena-Bustillos and Krochta 1994). The fruit is also particularly susceptible to postharvest chlorophyll degradation (Kader 1983), water loss (Avena-Bustillos and Krochta 1994) and chilling injury (Carvajal *et al.* 2011).

With the current consumer trend that favours pesticide-free fresh produce; the need for environmentally friendly treatments has become important. The main objective of the present study was to evaluate the effect of six different optimized

dipping treatments on postharvest quality retention of fresh repacked zucchini. In addition, the microbial load was determined at different postharvest stages to establish parameters at which fresh zucchini are still acceptable for consumption. The treatments used in this study were selected based upon their efficacy previously reported in the literature on similar food crops (cucumber, bell pepper and tomato) (El Ghaouth 1997; Lurie 1998; Liu 2007).

2. Materials and Methods

2.1. Fruit

Freshly harvested zucchini from the same cultivar (medium green variety) were obtained from the fresh produce market in Tshwane, Gauteng, South Africa. The fruit originated from the same producer, were freshly picked and packed under commercial conditions the day before and transported to the market in a refrigerated truck. Nine hundred and sixty fruit were randomly selected on the basis of size (10-12 mm in length, 6-8 mm in diameter), shape and quality. All fruit were briefly rinsed in sterile water to remove sand or other particles before treatment and left to air dry.

2.2. Treatments

2.1.1. Aloe vera

Commercial *Aloe vera* concentrate was purchased from a local pharmaceutical retail chain store and was prepared in a 1:3 ratio (*Aloe vera* concentrate: sterile distilled water) according to Martinez-Romero *et al*. (2006). The zucchini fruit were dipped in the *Aloe vera* solution for 5 min at 25°C and left to air-dry (10 min).

2.1.2. Chitosan

A 0.5% chitosan solution was prepared by dissolving chitosan (Sigma, C3646-500G) obtained from deacetylated chitin (crab shells, 75% deacetylated) in a 0.25% glacial acetic acid (Merck, Johannesburg, South Africa) solution (Romanazzi *et al.* 2001) under moderate stirring at approx. 50°C for 4 hrs. When dissolved, the pH of the solution was adjusted to 6 using a 0.1 M NaOH solution. The zucchini fruit were dipped in the chitosan solution at 25°C, immediately removed and allowed to air dry.

2.1.3. Warm water

The fruit were dipped in warm water (40°C) for 10 min as described by Lurie (1998) and allowed to air dry.

2.1.4. Citric acid / Ascorbic acid / $CaCl_2$

Solutions of 0.5% citric acid (Unilab, Krugersdrop, South Africa), ascorbic acid (Unilab) and $CaCl_2$ (Merck) were prepared separately by dissolving the respective powders in sterile distilled water (Lucera *et al.* 2010). Two groups of zucchini were dipped in each treatment solution respectively for 5 min at 25°C and left to air dry.

The fruit were randomly organised into groups of 10 fruit that were subjected to a treatment protocol before being packed in unperforated polypropylene clamshell containers (240 x 160 x 75 mm) (Marcoware, Marco Plastics, Alberton, South Africa) and stored at 5.8°C and 85% RH in darkness (Martinez-Romero *et al*. 2006) for up to two weeks. The control consisted of an acetic acid solvent control (refer to section 2.1.2) and an untreated control. Physiological, sensory and visual assessments were performed on the day of treatment, day seven and 14 of storage. Disease decay was noted if present on any treatment. One randomly selected zucchini per container was used for sensory evaluation and three for physiological assessment. The entire experiment was repeated.

2.3. Physiological assessment

2.3.1. Texture analysis

Flesh firmness was determined using a fruit firmness tester (TR, Italy). For each treatment, two zucchinis were tested for both the repeat experiments. One cm^2 of the peel was removed from opposite sides of the equatorial plane of each fruit and the penetration force was measured individually and recorded using a 2 mm probe. The recorded value was based on the force required to shear one zucchini and was expressed in kilograms.

2.3.2. Brix (total soluble solids)

For each treatment of the two repeat experiments, two zucchinis were used. The fruit were finely sliced into pieces and subsequently homogenized with a food processor (Phillips, Netherlands). The homogenised product was filtered through a fine sieve

and an aliquot of filtrate was used to determine the total soluble solids (TSS) value with a digital refractometer (Atago Co., Tokyo, Japan).

2.3.3. pH

Approximately 20 g of homogenised product was mixed with a 0.09% NaCl (Merck) solution and stomached (Seward Stomacher® 400 Circulator, Lasec, South Africa) at 230 rpm for 30 sec. The pH was measured after stomaching.

2.3.4. Weight loss

In order to determine whether any weight loss occurred during storage, both the treated and untreated zucchini fruit were weighed on the first day directly after treatment and subsequently at day 7 and 14 during the trial period.

2.4. Surface colour

The surface colour was tested using two zucchinis per replicate per treatment. Measurements were taken along the "equatorial axis" (opposite sides) using a Minolta Chromameter (model CR-300; Osaka, Japan). Results were expressed as L*, b* and a* values.

2.5. Visual quality

Decay incidence was visually assessed, and was recorded as a value between 1 and 4. Assessments were made according to the following criteria (Alvaro *et al*., 2009):

1 = <25% area affected by spoilage, 2 = between 25-50% affected,

3 = between 50-75% affected, 4 = >75% affected.

2.6. Sensory evaluation

For the sensory evaluation, whole zucchinis were thoroughly washed with water, ends were removed and zucchinis were cut in half. Sixteen portioned zucchinis (one half per panellist) were boiled over steam for five minutes using an Anvil Bain Marie (Model BMA 0002). Eight trained panellists (Department of Food Science, Sensory Research Division) were first presented with uncooked zucchini samples and instructed to rate these for uncooked attributes. After rating the uncooked zucchinis, samples were cooked and once again presented to each panellist for further evaluation. Panellists rated the zucchinis on a structured ten-point scale (e.g. 0 = not

intense; 10 = very intense) using attributes such as aroma intensity, skin colour, skin firmness, juiciness, sweetness, bitterness, sourness and aftertaste. The samples were evaluated over a two-week period at three intervals: days 0, 7 and 14.

2.7. Microbial evaluation

For the microbial evaluation, four zucchinis per treatment for both the repeat experiments were handled as follows: zucchinis were washed for 1 min (optimised time, unpublished data) in a Eumax ultrasonic cleaner (Labotec, Johannesburg) with 1 L Ringer's (Merck) solution supplemented with Tween 80 CP (Merck) to remove surface microflora. The Ringer's solution was filtered through a 45 μm membrane (National Seperation, Johannesburg) using a vacuum pump (Balzers, Germany), after which the membrane was transferred to 9 ml Peptone buffered water (PBW) (Merck), vortexed and standard serial dilutions made. Samples were prepared in duplicate, and 100 μl from each dilution was plated out onto Standard 1 Nutrient Agar (STD1) and Malt Extract Agar (MEA) (Biolab, Johannesburg). The STD1 and MEA plates were incubated for 2 and 4 days respectively at 25°C before counting colonies and determining microbial load expressed as log cfu/cm^2.

2.8. Statistical evaluation

Each experiment was designed as a randomised complete block design, containing two replicates; each replicate consisting of 240 fruit, and the entire experiment was performed in duplicate. Physiological, surface colour and microbiological data were analysed by one-way analysis of variance using General Linear Model (GLM) test to determine significant differences between treatments (SAS Software Version 9.3). Sensory data were captured and analysed using Compusense® five data software (Compusense® 5, release 5.2, Compusense Inc., Guelph, ON, Canada) and Statistica version 10 (Statsoft 2011). Repeated measures in Analysis of Variance (ANOVA) of a GLM was used to determine the effects of treatment over storage time and the interaction effect on the sensory properties of the zucchini samples. The storage time was treated as the repeated measures variable, three storage periods (day 0, day 7 and day 14). The Fisher Least Significance Difference (LSD) test was used to investigate the nature of the differences.

3. Results and Discussion

3.1. Physiological parameters

The *Aloe vera* and $CaCl_2$ treated samples were more effective in maintaining flesh firmness after the 14-day trial period, in contrast to the control which showed a decline in flesh firmness after the first 7 days of the trial period (Figure 1b) . Calcium treatments have been shown to retain the firmness and quality of vegetables and fruit as Ca contributes to the structural integrity of plant cell walls and membranes (Izumi and Watada 1995). The *Aloe vera* treatment was also more effective in maintaining flesh firmness as was reported by Martinez-Romero *et al*. (2006). The active compound in *Aloe vera* has hygroscopic properties, which enables the formation of a barrier to water diffusion between fruit and the environment (Martinez-Romero *et al*. 2006). In contrast to findings by Lurie (1998) and Ansorena *et al.* (2011), flesh firmness was maintained for the first seven days after a warm water treatment. The firmness of the zucchini subjected to the chitosan treatments was significantly different from the non-treated and solvent control treatments, indicating its potential to prolong shelf life.

The total soluble solids (TSS) value remained the lowest for the warm water treated samples throughout the trial. The values were the greatest for the *Aloe vera* and $CaCl_2$ samples at seven and 14 days (Figure 1c) respectively, which indicates a more ripened development stage for both of these treatments. The ascorbic acid treatment showed the greatest increase in TSS from day 0 to day 14. The control showed comparable performance to that of the acetic acid treatment, with a moderate increase from day 0 to day 7 and then a slight decrease in TSS from day 7 to day 14. The TSS value was the lowest for the warm water treatment that indicated less evident ripening progression. Hot water dips have previously been successfully used to improve the shelf-life of vegetables and fruit (Lurie 1998; Ansorena *et al.* 2011).

The pH values increased from day seven to 14 for all the samples over the storage time, but remained in the $6.0 < pH < 6.4$ range. Wills and Widjanarko (1995) similarly reported that the pH of papayas increases over storage time as the amount of fruit acids decline. After seven days of storage, the pH was the highest for $CaCl_2$, and the lowest for the *Aloe vera* and control samples, with the greatest decline in pH over the experimental period shown by $CaCl_2$ treated samples (Figure 1d).

Weight loss was the highest for the untreated control samples after seven days of storage, while there were no significant weight loss differences between any of the treatments after 14 days of cold storage (Figure 1a).

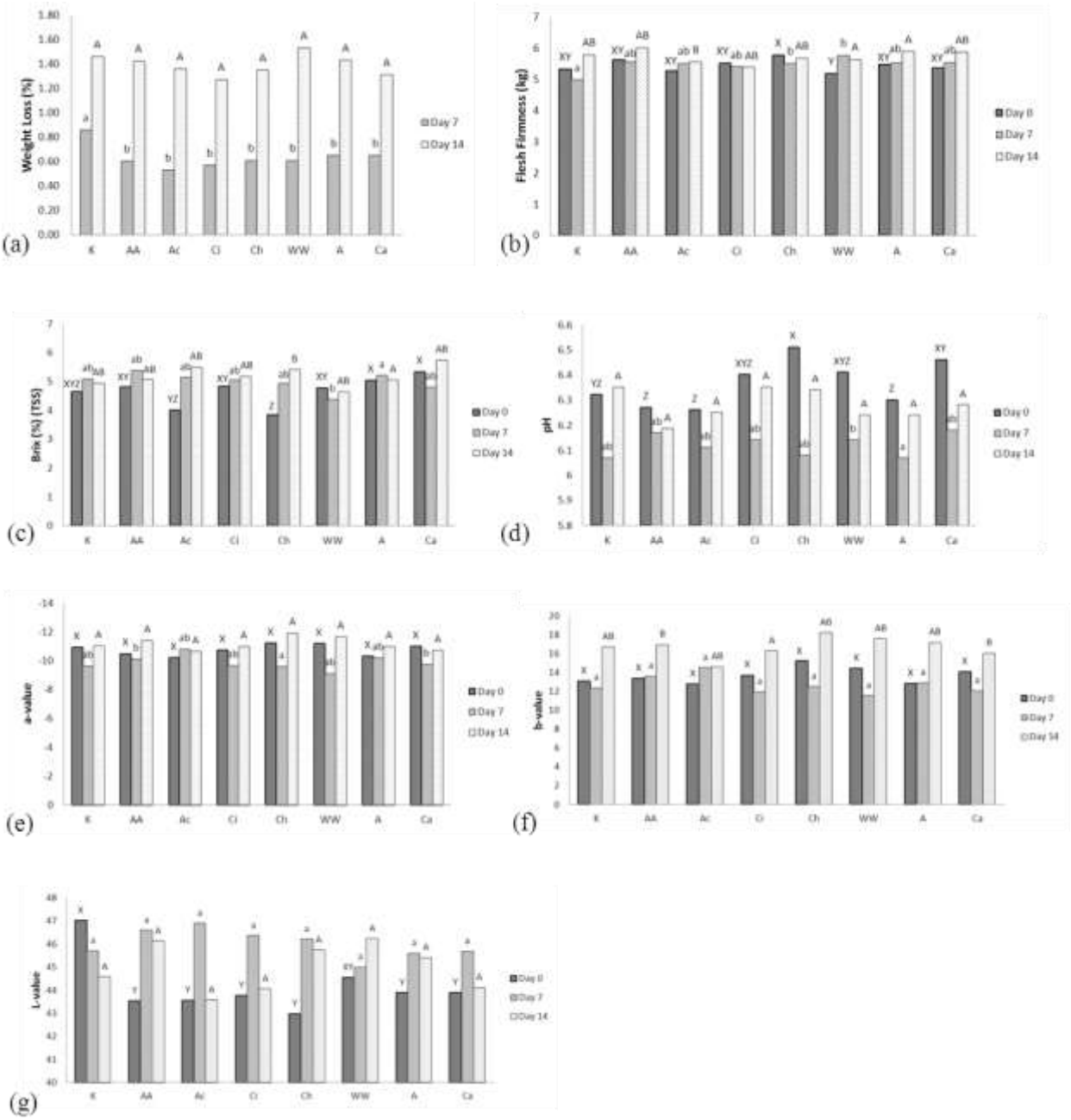

Figure 1. Effect of different treatments after 7 and 14 days (5.8°C) (85% relative humidity) on (a) Weight loss, (b) Flesh firmness, (c) Brix, (d) pH, (e) L-values, (f) a-values and (g) b-values of repacked zucchini. Means with the same letter are not significantly different at $P < 0.05$ by Fisher's Least Significant Test (K = Control, AA = Acetic acid solvent control, Ci = Citric Acid, Ac = Ascorbic acid, Ch = Chitosan, A = *Aloe vera*, Ca = $CaCl_2$, WW = Warm water).

3.2. Surface colour

With respect to surface colour, the L-value (Figure 1g) for dip treatments did not differ significantly. The untreated control samples showed the greatest loss in L-values over the experimental period. The ascorbic acid treatment retained the zucchins' green colour significantly better than the warm water (a-value interpretation, Figure 1e) and chitosan (b-value interpretation, Figure 1f) treated samples at seven and 14 days of storage respectively. Citric - and ascorbic acids are traditionally used to prevent enzymatic browning of freshly cut fruit and vegetables (Toivonen and Brummel 2008).

3.3. Visual quality

In this study, none of the fruit exhibited spoilage of more than 25% of the total surface area per zucchini at a significance level of $p < 0.05$. General decay in the isolated cases were mostly caused by Rhizopus rot (data not presented).

3.4. Sensory evaluation

Compared to the control groups, the eight panelists could not distinguish significant sensory property changes between the six zucchini treatment groups during the two-week storage period at a significance level of $p < 0.05$ (data not presented).

3.5. Microbial counts

Figure 2 shows that on the day of treatment application, the total microbial (mesophilic aerobic bacteria, filamentous fungi and yeasts) load of the control and treated zucchini ranged between 1.9 and 6.3 log cfu/cm^2 on the day of treatment. In both control and treated samples, a significant increase in microbial load was observed over 14 days of cold storage at 5.8°C, although the increase was not significant for the *Aloe vera* and warm water samples after seven days. The highest increase in microbial load from the day of treatment to day seven was observed for the warm water, citric acid and chitosan treated samples with increases of 3.2, 3.3 and 3.7 log cfu/cm^2 respectively. The amount of total microbial load increase for all the samples from day seven to 14 was less than the amount of increase from day zero to day seven, except for the untreated control samples that showed a higher escalation in average microbial counts. The highest microbial count of 10.4 log cfu/cm^2 was noted for the solvent control samples after the two-week trial period.

It has previously been reported that calcium has an inhibitory effect on the growth of microorganisms (Izumi and Watada 1995). Calcium is known to maintain the structural integrity of cell walls and membranes, thereby possibly providing resistance to microbial infection (Conway and Sams 1984). While the antimicrobial effect of *Aloe vera* and warm water was not evident in the results presented for the total microflora, it was noted that it specifically showed anti-yeast properties from day seven to day 14 (yeast counts decreased). Chitosan is an edible polymer coating that has antifungal properties through increasing their susceptibility to lytic enzymes (Ali *et al.* 2012), but also addresses two prime factors that influences quality of zucchini, i.e. water loss and high respiration rates. In this study, the antimicrobial effect of chitosan was evident on the day of the treatment and it maintained a lower microbial load throughout the 14-day trial compared to the other treatments.

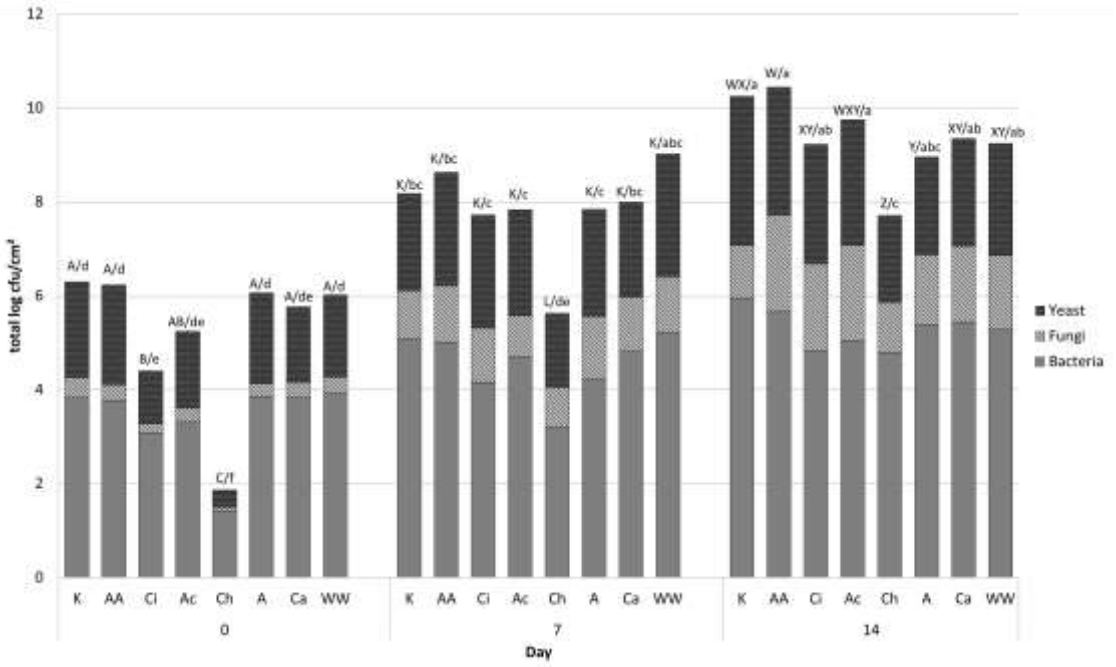

Figure 2. Average microbial total load of treated and untreated zucchini during 14 days of storage. Uppercase letters that are dissimilar indicate significant differences within day 0, 7 or 14. Lowercase letters that are dissimilar indicate significant differences over the 14 day period (K = Control, AA = Acetic acid solvent control, Ci = Citric Acid, Ac = Ascorbic acid, Ch = Chitosan, A = *Aloe vera*, Ca = $CaCl_2$, WW = Warm water).

4. Conclusion

To the best of our knowledge, this is the first report where $CaCl_2$, *Aloe vera*, warm water, chitosan, ascorbic- and citric acid dipping treatments were compared for maintaining the quality of fresh repacked zucchini.

The use of *Aloe vera* and $CaCl_2$ may be appealing environmentally friendly treatments for practical application in the industry. Since the stand-alone treatments in this study exhibited potential for effective quality maintenance of repacked zucchini, combination treatments should be carefully explored. It is also recommended that similar studies be conducted on a commercial basis.

Product quality is often considered acceptable with mesophilic bacterial counts of 10^2 to 10^6 cfu/g (Nguyen-the and Carlin 1994). In this study, the highest microbial count (10 log cfu/cm^2) was found on the zucchini surface of the control samples after the two-week trial period. Of note was the fact that these zucchinis were still in a pallatable state and considered of high visual and sensory quality. Barriga *et al.* (1991) found that a total number of 10^7 cfu/g psychrotrophic microorganisms on shredded lettuce still warranted a product with a high visual quality rating. Abusive

temperatures or rough handling may rather be the cause of product deterioration instead of high microbial loads (Zagory 1999). The value of using a generic total microbial load for fresh fruit and vegetables can be debated in the light of new emerging scientific information. The South African Department of Health's guidance document for ready-to-eat food, specifies fresh fruit and vegetables not to exceed a total yeast and fungal count of <100 000/g to ensure that the product is safe for consumption (Department of Health Directorate: Food Control 1997). The referred document does not stipulate guidance regarding bacterial loads and provides no microbial load recommendations for harvested ready-to-eat fresh produce. As a separate outcome of this study, we would like to suggest that a normal healthy microbial load of fresh – and mature packed zucchini could be in the order of 6-10 log cfu/cm^2. Further research should focus on establishing a microbial load on fresh zucchini at the point of harvest and further down the food chain.

5. References

ALI, A., MOHAMED, M.T.M. and SIDDIQUI, Y. 2012. Control of Anthracnose by chitosan through stimulation of defense-related enzymes in Eksotika II papaya (*Carica papaya* L.) fruit. JBLS *3*, 114-126.

ALVARO, J.E., MORENO, S., DIANEZ, F., SANTOS, M., CARRASO, G. and URRESTARAZU, M. 2009. Effects of peracetic acid disinfectant on the postharvest of some fresh vegetables. *J Food Eng. 95*, 11-15.

ANSORENA, M.R., MARCOVICH, N.E. and ROURA, S.I. 2011. Impact of edible coatings and mild heat shocks on quality of minimally processed broccoli (*Brassica oleracea* L.) during refrigerated storage. *Postharvest Biol Tec. 59*, 53-63.

AVENA-BUSTILLOS, R. and KROCHTA, M.J. 1994. Optimization of edible coating formulations on zucchini to reduce water loss. *J Food Eng. 21*, 197-214.

BARRIGA, M.I., TRACHY, G., WILLEMOT, C. and SIMARD, R.E. 1991. Microbial changes in shredded iceberg lettuce stored under controlled atmospheres. *J Food Sci. 56*, 1586-1588.

CARVAJAL, F., MARTINEZ, C., JAMILENA, M. and GARRIDO, D. 2011. Differential response of zucchini varieties to low storage temperature. *Sci Hort. 130*, 90-96.

CHOI, S.W. and SAPERS, G. 1994. Effects of washing on polyphenols and polyphenol oxidase in commercial mushrooms (*Agaricus bisporus*). *J Agric Food Chem. 42,* 2286-2290.

CONWAY, W.S. and SAMS, C.E. 1984. Possible mechanisms by which postharvest calcium treatment reduces decay in apples. *Phytopathology 74,* 208-210.

DEPARTMENT OF HEALTH DIRECTORATE: FOOD CONTROL. 1997. Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. *Regulations Governing Microbiological Standards for Foodstuffs and Related Matters. R.692* of 16 May.

EL GHAOUTH, A., ARUL, J., WILSON, C. and BENHAMOU, N. 1997. Biochemical and cytochemical aspects of the interactions of chitosan and *Botrytis cinerea* in bell pepper fruit. *Postharvest Biol Tec. 12,* 183-194.

ESQUINAS-ALCAZAR, J.T. and P.J. GULICK. 1983. *Genetic Resources of Cucurbitaceae,* IBPGR, Rome.

FAOSTAT 2014. Food and Agricultural Organization of the United Nations http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E (accessed May 19, 2014).

HEUN HONG, J. and GROSS, K. C. 1998. Surface sterilization of whole tomato fruit with sodium hypochlorite influences subsequent postharvest behavior of fresh-cut slices. *Postharvest Biol Tec. 13,* 51-58.

IZUMI, H. and WATADA, A.E. 1995. Calcium treatment to maintain quality of zucchini squash slices. *J Food Sci. 60,* 789-793.

KADER, A. 1983. Postharvest quality maintenance of fruits and vegetables in developing countries. In *Postharvest Physiology and Crop Preservation,* (M. Lieberman, ed.) pp. 455-469, New York.

KAMI, D., MURO, T. and SUGIYAMA, K. 2011. Changes in starch and soluble sugar concentrations in winter squash mesocarp during storage at different temperatures. *Sci Hort. 127,* 444-446.

LIU, J., TIAN, S., MENG, X. and XU, Y. 2007. Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. *Postharvest Biol Tec. 44,* 300-306.

LUCERA, A., COSTA, C., MASTROMATTEO, M., CONTE, A. and DEL NOBILE, M.A. 2010. Influences of different packaging systems on fresh-cut zucchini (*Cucurbita pepo*). *Innov Food Sci Emerg. 11,* 361-368.

LURIE, S. Postharvest heat treatments of horticultural crops. 1998. *Hort Reviews 22*, 91-121.

MARTINEZ-ROMERO, D., ALBURQUERQUE, N., and VALVERDE, J.M. 2006. Postharvest sweet cherry quality and safety maintenance by *Aloe vera* treatment: a new edible coating. *Postharvest Biol Tec. 39,* 93–100.

NGUYEN-THE, C. and CARLIN, F. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit Rev Food Sci Nutr. 34,* 371-401.

RAUTENBACH, F. and VENTER, I. 2010. Hydrophilic and lypophilic antioxidant capacity of commonly consumed South African fruits, vegetables, grains, legumes, fats/oils and beverages. *J Food Comp Anal. 23,* 753-761.

ROMANAZZI, G., NIGRO, F., IPPOLITO, A. and SALERNO, M. 2001. Effect of short hypobaric treatments on postharvest rots of sweet cherries, strawberries and table grapes. *Postharvest Biol Tec. 22,* 1-6.

TOIVONEN, P.M.A. and BRUMMELL, D.A. 2008. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biol Tec. 48,* 1-14.

WILLS, R.B.H. and WIDJANARKO, S.B. 1995. Changes in physiology, composition and sensory characteristics of Australian papaya during ripening. *Aust J Exp Agr. 35,* 1173-1176.

ZAGORY, D. 1999. Effects of post-processing handling and packaging on microbial populations. *Postharvest Biol Tec. 15,* 313-321.

Acknowledgements

This research was financially supported by the Gauteng Department of Agriculture and Rural Development (GDARD). The authors would like to thank the Department of Food Science at the University of Pretoria for the sensory analysis. We are also thankful to Trish Beart, Quenton Volschenk and Francois Duvenage for their assistance.