Accepted Manuscript

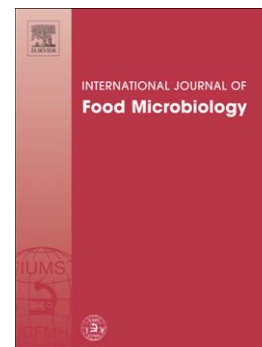# ORGANIC ACID BASED SANITIZERS AND FREE CHLORINE TO IMPROVE THE MICROBIAL QUALITY AND SHELF-LIFE OF SUGAR SNAPS

Sam Van Haute[a,b], Mieke Uyttendaele[a], Imca Sampers[b]

[a]Laboratory of Food Microbiology and Food Preservation, Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium

[b]Laboratory of Food Microbiology and Food Biotechnology, Department of Industrial Biological Sciences, Faculty of Bioscience Engineering, Ghent University Campus Kortrijk, Graaf Karel de Goedelaan 5, 8500 Kortrijk

***Corresponding author.** Corresponding author: Mailing address: Laboratory of Food Microbiology and Food Biotechnology, Department of Industrial Biological Sciences, Faculty of Bioscience Engineering, Ghent University Campus Kortrijk, Graaf Karel de Goedelaan 5, B-8500 Kortrijk, Belgium. Phone: +32 56 24 12 11. Fax: +32 56 24 12 24. Electronic mail address: imca.sampers@ugent.be

ABSTRACT

A screening in a sugar snaps packaging company showed a converged build-up of aerobic psychrotrophic plate count (APC) (ca. 6.5 log CFU/100 mL), yeasts and molds (Y&M), and lactic acid bacteria (LAB) (both ca. 4.5 log CFU/100 mL) in the wash water in the absence of water sanitizer, and a low build-up of chemical oxygen demand (30 ± 5 mg O_2/L) and turbidity (5.2 ± 1.1 NTU).

Decontamination experiments were performed in the lab with Purac FCC 80® (80% L(+) lactic acid), two other commercial water sanitizers based on organic acids (Natraphase-ABAV®, and Natraphase-FVS®) and chlorine to evaluate their performance in reduction of the sugar snaps microbial load as well as their functionality as disinfectant of the wash water to avoid cross-contamination.

An additional 1 log reduction of APC on the sugar snaps was achieved with lactic acid in the range 0.8 to 1.6 %, ABAV 0.5 %, and free chlorine 200 mg/L when compared to a water wash, while no significant difference in the numbers of Y&M was obtained when washing in sanitizer compared to water. There was no significant influence of the studied concentration and contact time on decontamination efficiency. Treatment with lactic acid 0.8% resulted in a lower APC contamination on the sugar snaps than on the untreated and water washed samples for 10 days. Chlorine 200 mg/L was the only treatment able to maintain the Y&M load lower than the untreated samples throughout the entire storage duration. The use of water sanitizers could not extend the sensorial shelf-life. Microbial loads were not indicative/predictive for visual microbial spoilage (shelf-life limiting factor), whereas maturity and amount of damage at the calyx end of the pods were.

2

The APC wash water contamination (5.2 log CFU/100 mL) was reduced significantly by chlorine 20 to 200 mg/L (to 1.4 log CFU/100 mL), ABAV 0.5 to 1.5 % (to 2.7 log CFU/100 mL), FVS 0.5 % (to 2.7 log CFU/100 mL) and lactic acid 0.8 to 1.6% (to 3.4 log CFU/100 mL). Only the use of chlorine enabled the reduction of the Y&M wash water contamination significantly (from 3.4 to 1.4 log CFU/100 mL). The low physicochemical build-up of the sugar snaps wash water during the industrial washing process makes free chlorine attractive as water disinfectant to prevent bacterial and fungal cross-contamination, whereas the sanitizers based on organic acids are not, due to their weak water disinfection efficiency.

1. INTRODUCTION

Most grown sugar snaps (*Pisum sativum* var. *macrocarpon*) in the world are produced for local markets. In the last decades however, there has been a rise in the production of non-traditional export crops, including sugar snaps. Industrialized countries import large quantities of sugar snaps from tropical developing countries (such as Kenya and Guatemala), in order to have a year round supply and because of the high labor costs involved with picking (Humphrey et al., 2004; Messiaen et al., 2004). The main spoilage microorganisms on beans and peas are *Pythium butleri*, the fungal plant pathogens *Rhizoctonia solani*, *Sclerotinia* spp, and *Botrytis cinerea* and the pectinolytic bacterium *Erwinia carotovora* that break down the pectic substances of the middle lamella, with consequential loss of mechanical protection and rigidity (Brummell, 2006; Tournas, 2005; Walker et al., 1998). The production of acid or antimicrobial compounds by native microbial flora may interfere with the colonization, survival and proliferation of foodborne pathogens (a.o. *Salmonella* spp.*,* pathogenic *Escherichia coli, Listeria monocytogenes*) (Johnston et al., 2009; Liao and Fett, 2001; Shi et al., 2009; Teplitski et al., 2011). On the other hand, the chances of bacterial pathogen

proliferation on and internalization in fresh produce are improved by the disruptive actions of certain fungal and bacterial spoilage microorganisms on the plant tissues (Brandl & Sundin, 2013; Critzer and Doyle, 2010; Ryser et al., 2009). Sugar snaps from Guatemala, which can be consumed either raw or cooked, have been the suspected vector of a *Shigella dysenteriae* outbreak in Sweden (May-June 2009) (Lofdahl et al., 2009) and a second *Cyclospora cayetanensis* outbreak (June 2009) was also reported in Sweden, with sugar snaps from Kenya as the suspected source (Insulander et al., 2010).

Organic acids applied at relatively low concentrations exhibit inhibitory effects on microbial growth and are used to preserve acid foods and beverages. At higher concentrations organic acids can be used as decontaminants of food products such as fruits and vegetables and meat carcasses to improve food safety and quality (Virto et al., 2006). Organic acids are weak acids, and therefore they exist in a pH dependent equilibrium between the dissociated and undissociated state. The uncharged, undissociated acid can diffuse across the plasma membrane of microorganisms. Inside the cell the organic acid deprotonates, causing a pH drop and accumulation of toxic anions. As such, membranes can be disrupted, the proton motive force dissipated, essential metabolic reactions inhibited, and the intracellular pH homeostasis stressed (Brul and Coote, 1999; Capozzi et al., 2009).

Chlorine is the most used water disinfectant in fresh produce washing processes because of the low cost, the proven ability to rapidly inactivate suspended bacteria, and the minimal impact on the nutritional and sensorial fresh produce quality. Drawbacks of chlorination are the possibility of chlorine gas generation in the working environment when incorrectly applied (i.e. below pH 5 and excessive dosing), the rapid decomposition in the presence of organic matter, and most notoriously the possibility of creating harmful disinfection by-

4

products in the wash water. However, studies on uncut carrots and fresh-cut lettuce have shown that only negligible or undetectable amounts of disinfection by-products were detected on the final product when a final rinse with tap water is applied (Klaiber et al., 2005; Van Haute et al., 2013).

After transport by airplane or container ship, sugar snaps are washed for rehydration and removal of materials from the pod surface. The objective of this study was to evaluate the use of water sanitizers for the reduction of the sugar snaps microbial load and extension of shelf-life as well as their functionality as disinfectant of the wash water to prevent cross-contamination. To the knowledge of the authors, hitherto, no studies have been published regarding the use of water disinfectants to improve the microbial quality of sugar snaps, extend the shelf-life and maintain the wash water quality of sugar snaps wash water. As decontamination efficiency depends in part on the produce surface and the way microorganisms attach to it, i.e. presence of stomata, surface roughness and hydrophobicity of the produce (Gomez-Lopez et al., 2008), the lack of knowledge on decontamination of pod vegetables (especially of snow peas and sugar snaps which can be consumed raw) makes sugar snaps decontamination a topic of interest. In this study, an on-site screening of an industrial sugar snaps washing process in the absence of water sanitizers was performed in a packaging company to observe the evolution of microbial and physicochemical parameters in function of processing time. In a second step, lab-scale experiments were performed with commercial formulations based on organic acids as produce and wash water sanitizers, and with chlorine as reference method.

2. MATERIALS AND METHODS

2.1. The sugar snaps

The sugar snaps used in the experiments originated from Peru and Guatemala. They were transported to the packaging company by container ship during 19 to 22 days at 3 ± 1 °C in modified atmospheric packaging ($O_2 < 10\%$, $CO_2 > 0.5\%$, Xtend®, StePac, Israel). Experiments were performed on 5 different batches (1 from Peru for the evaluation of the washing process in the packaging company and 4 for the decontamination experiments, of which 3 from Peru and 1 from Guatemala) that were sampled at different dates in the period October – December 2012. The acquired sugar snaps for the decontamination trials were as they were delivered to the packaging company, i.e. unwashed in crates of 4.5 kg.

The state of the pod at the calyx end, the amount of mechanical damage on the sugar snaps and the size of the seeds in the pod, were compared among batches at reception, in order to be able to observe the impact of these characteristics on microbial number, growth, and the onset of visual microbial spoilage. Seed size is an indicator of maturity status. In the immature state, seeds do not fill the hull, in the mature state they fill the hull without deforming it, and in the overmature state they deform the hull (Basterrechea and Hicks, 1991).

2.2. Evaluation of the washing process in the packaging company

The packaging company applied a bubble washer of 750 L volume with a replenishing rate of 400 L/h. 1000 kg of sugar snaps were washed in 188 minutes, air dried, screened with machine vision to remove pods showing excessive browning, and packaged in 300 g consumer units. At several time points during the washing process, samples of both sugar snaps before and after washing (after 0, 18, 54, 96, and 188 minutes) and of the wash water (after 0, 10, 18, 30, 54, 96, 120, 188 minutes) were taken. Also, samples of the tap water were taken at the point of entrance in the washing bath. Temperature, pH, and conductivity (all with HQ40d meter, Hach Lange, Belgium) of the wash water were measured at the packaging

company. The residence time of the sugar snaps in the washing bath was measured (n=12) by labeling individual sugar snaps with fluorescent tape and timing the period from entrance till exit from the washing bath. The samples were transported under refrigerated conditions to the lab for further analysis. Alkalinity, turbidity, chemical oxygen demand (COD) of the wash water were determined. Water samples were also analyzed for aerobic psychrotrophic plate count (APC), yeasts and molds (Y&M), and lactic acid bacteria (LAB). Sugar snaps, collected before and after washing, were analyzed for moisture content and water activity (a_w). Samples of the sugar snaps (250 g) were stored in plastic bags for 22 days at 5 ± 1 °C under normal atmospheric conditions and periodically sampled (after 0, 6, 10, 15, and 22 days) for APC, Y&M, and LAB and judged for onset of visual microbial spoilage (i.e. fungal rot, bacterial slime formation).

2.3. Evaluation of water sanitizers to improve shelf-life of sugar snaps and maintain wash water quality

Sodium hypochlorite (28.4 g/L NaOCl, La Croix, Belgium), acetic acid (Sigma-Aldrich, Belgium), Purac FFC 80® (80% L(+) lactic acid, Purac, The Netherlands), Natraphase-ABAV® (fine powder containing natural acids, Natural Biotechnology, Belgium) or Natraphase-FVS® (blend of EU and FDA food approved organic acids and vitamins, Natural Biotechnology, Belgium) were used as water disinfectants. The experimental disinfectant concentration - contact time settings are shown in Table 1. For Purac FFC 80, the added concentration is expressed as active compound, i.e. L(+) lactic acid. For chlorine, the pH was adjusted to 6.5 using HCl (1 M). Each experiment (i.e. disinfectant; concentration; contact time) was executed on three different batches in order to incorporate possible influence of variation in microbiology and physical and physiological differences among sugar snaps in different batches.

Portions of 250 g of sugar snaps were washed by mechanical agitation in 4 L of tap water (5 ± 1 °C) with added water disinfectant. After washing, water samples were immediately quenched with $Na_2S_2O_3$ (0.1 M) for quenching sodium hypochlorite, or phosphate buffer (pH 7.5) for Purac FCC 80, acetic acid, ABAV and FVS. Microbial analyses (APC, Y&M, LAB) were performed on the water samples. The sugar snaps were rinsed (0.1 L/kg.s for 10 s) with tap water. At the highest exposure conditions in the experiment (i.e. highest contact time and disinfectant concentration), samples of washed sugar snaps were either rinsed or not rinsed to observe the effect of residual disinfectant on discoloration, off-odors, damage and texture loss. ABAV samples were never rinsed and FVS samples were always rinsed because these patented formulations were recommended to be used respectively with or without a final rinsing step by the manufacturers. All samples were dried with sterile absorbent paper, and subsequently, samples were screened for discoloration, and sugar snaps showing browning were discarded. The samples were stored in plastic bags at 5 ± 1 °C for 22 days under normal atmospheric conditions and periodically sampled (after 0, 6, 10, 15, and 22 days) for microbial analyses (APC, Y&M, LAB), and at the same time monitored for the presence of visual microbial decay, discoloration, off-odors, damage and texture loss due to the decontamination treatments.

2.4. Physicochemical parameters

Alkalinity was determined with acid titration, turbidity with a turbidimeter (HI98703; HANNA Instruments; Belgium), chemical oxygen demand (COD) according to the small-scale sealed-tube method (LCI 400; Hach Lange; Belgium), absorbance at UV 254 nm with a UV-Visible spectrophotometer (UV1601, Shimadzu, Belgium) and quartz cuvettes with a 1-cm path length (Hellma, Belgium) after filtration through a 0.45 µm polytetrafluorethylene

filter (Macherey-Nagel, Belgium). a_w of the sugar snaps was measured with a dew point water activity meter (AquaLab Series 4:4TE, Decagon Devices, The Netherlands). Moisture content of the sugar snaps was determined through homogenization of 5 g of sample (T18 Basic Ultra-Turrax, IKA, Germany) and drying in an air circulation oven of 105 °C for 3 hours. Free chlorine was measured with the N,N-diethyl-p-phenylenediamine (DPD) colorimetric method (Eaton et al., 2005).

2.5. Microbial analyses

The sugar snaps samples were prepared by weighing 25 g of sugar snaps in a sterile stomacher bag with full-surface filter (0.5 mm pore size) (VWR, Belgium) which was homogenized in 225 mL peptone water (Oxoid, Belgium) for 1 min. Sugar snaps were analyzed for APC, Y&M, and LAB. APC was enumerated with plate count agar (Oxoid, Belgium) using the pouring plate method (incubation at 22 °C, 5 days). Y&M were enumerated with Rose Bengal Chloramphenicol agar (Oxoid, Belgium) containing 150 mg/L chloramphenicol and using the spreading plate method (incubation at 22 °C, 5 days). Membrane filtration was used to lower the detection limit of Y&M to 1 log CFU/g. LAB were enumerated with MRS (De Man, Rogosa, Sharpe) agar (Oxoid, Belgium), containing 1.4 g/L sorbic acid and with a final pH of 5.7, adjusted with NaOH (1 mole/L), using the pouring plate method with an additional cover layer of agar (incubation at 22 °C, 5 days). The water samples were analyzed for the same microorganisms, using the same enumeration methods. In addition, membrane filtration of 10 or 100 mL water was used to lower the detection limit for microbial enumeration to respectively 1 or 0 log CFU/100 mL.

2.6. Statistics

Data analysis was performed with SPSS statistics 21. Influence of disinfectant type, concentration and contact time was assessed with one-way ANOVA, or Brown-Forsythe when equal variance could not be assumed. Group comparison was done with post-hoc tests (Tukey or Games-Howell) when all relations among groups were of interest. However, when only certain relations were of interest, i.e. a significant reduction of the wash water contamination, or a significantly lower contamination on the sugar snaps compared to water washed or untreated sugar snaps, simple contrast analysis was performed. A level of significance $p \leq 0.05$ was chosen for all statistical analyses.

3. RESULTS

3.1. Evaluation of the washing process in the packaging company

The average residence time of the sugar snaps in the washing bath was 26 ± 15 s. The microbial contamination in the wash water increased till about 25 min of exploitation after which the wash water contamination remained relatively stable (Figure 1). For APC and Y&M, the microbial contamination of the municipal water used in the washing bath immediately at the tap was significantly lower than the water samples taken in the washing bath immediately before start of the washing process, indicating presence of some microbial contamination on the washing equipment prior to start of operation. The microbial load on the sugar snaps before washing (3.0 ± 0.8, 2.7 ± 0.4, 2.3 ± 0.5 log CFU/g for APC, Y&M, and LAB respectively) was not significantly different from that on the washed sugar snaps (3.5 ± 0.4, 2.6 ± 0.4, 2.2 ± 0.8 log CFU/g for APC, Y&M, and LAB respectively). The microbial contamination on the washed sugar snaps did not change significantly in function of exploitation time. The COD and the turbidity of the wash water were significantly correlated (spearman's rho = 0.668; p = 0.005). Both became relatively stable from about an hour of exploitation until the end of operation: COD of 30 ± 5 mg O_2/L and turbidity of 5.2 ± 1.1

10

NTU (Figure 2). The temperature was 7.7 ± 0.7 °C, the pH 8.0 ± 0.1, the conductivity 425 ± 4 µS/cm, and the alkalinity 3.05 ± 0.02 mmole/L bicarbonate. All these parameters did not significantly change in function of processing time. The washing process increased the water content of the sugar snaps (from 81.6 ± 3.5 % to 86.0 ± 1.8 %, $p = 0.304$), though not significantly. Also, the a_w increased significantly (from 0.986 ± 0.001 to 0.990 ± 0.001, $p = 0.004$).

3.2. Microbial and visual quality of untreated sugar snaps at reception and during storage

The variation of APC and LAB among different batches of sugar snaps at reception and during storage was more pronounced than for the Y&M contamination (Figure 3). The onset of visual microbial decay was not directly related to the overall microbial contamination degree of the sugar snaps. As the LAB were below or close to the detection limit (1 log CFU/g) in some batches, it was hard to make statistical claims concerning disinfection of LAB and therefore no such conclusions were made. The initial seed size, and to lesser degree the integrity of the calyx end, seemed to have impact on the onset of visual microbial decay (Table 2). Violation of calyx end integrity became most apparent through brown discoloration and loss of firmness. The visual microbial decay manifested itself in the pod tissue towards the calyx end, and on major mechanical wounds. Except for batch 1 where all samples and most individual sugar snaps within samples showed signs of microbial decay, the onset of microbial decay in the other batches was mostly only visual on 1 sugar snap within a decaying sample (comprising ca. 20 - 50 remaining sugar snaps dependent on the storage time), which at a later date could become visible on one or more other sugar snaps.

3.3. Evaluation of water sanitizers to improve shelf-life of sugar snaps

Certain of the tested disinfectant settings caused damage to the sugar snaps, i.e. brown discoloration and formation of irregularities on the pod surface, more specifically, pit formation. In addition, acetic acid caused off-odors (Table 1). The data of the settings that caused damage to the product were not further incorporated in the shelf-life analyses. A water wash did significantly lower the concentration of Y&M (0.6 ± 0.4 log reduction; $p = 0.003$) on the sugar snaps, yet not of APC (0.5 ± 0.7 log reduction; $p = 0.059$) (Table 3). However, it is important to not blindly accept the statistical analysis. Reductions of 0.5 log compared to the untreated samples are very low in microbiological terms, both from the point of food spoilage (no considerable impact on the regrowth) and of food safety (the human dose-response and the associated increase in risk) (FDA, 2001). Duration of washing had no influence on reduction efficiency of any of the washing treatments, including a water wash, and these values were pooled to increase sample sizes for statistical analysis. Lactic acid in the range 0.8 to 1.6 % (1.4 ± 0.5 log reduction), ABAV 0.5 % (1.6 ± 0.2 log reduction), and free chlorine 200 mg/L (1.4 ± 0.5 log reduction) caused a significantly higher reduction of APC than a water wash (Table 3). In the studied concentration ranges, there was no relation between concentration and decontamination efficiency of APC for chlorine ($p = 0.648$) and FVS ($p = 0.759$) and some, yet no significant relation for ABAV ($p = 0.069$) and lactic acid ($p = 0.057$). None of the decontamination treatments removed Y&M significantly more effective from the sugar snaps than a water wash. Free chlorine (range 20 to 200 mg/L) had the highest reduction of Y&M (on average 1.0 ± 0.7 log reduction).

None of the treatments maintained the APC contamination lower than the untreated and water washed samples for the whole storage duration (Table 3). Treatment with lactic acid 0.8% resulted in a lower APC contamination on the sugar snaps than on the untreated or water washed samples for 10 days. Chlorine 200 mg/L was the only treatment able to maintain the

12

Y&M load lower than the untreated samples throughout the entire storage duration. For the other treatments, any significance in microbial reduction on the sugar snaps was lost in less than 10 days of storage.

Visual microbial decay occurred more or equally rapid on untreated than treated (including water washed) sugar snap samples (Table 4). Disinfection concentration had no effect on delaying the occurrence of visual microbial decay. In batch 1 and 2, the samples which showed microbial decay had a more rapid growth of APC than the other samples, and overall high counts were reached during storage (Figure 4). Batch 3 showed similar visual microbial decay as batches 1 and 2, but a relatively high proportion of batch 3 were Y&M, and no differences in counts between decayed and other samples were observed for Y&M or APC. Batch 4 also had a high relative abundance of Y&M, yet none of the samples of batch 4 showed any visual microbial decay during storage. The visual decay manifested itself in the same way as with the untreated samples. There was no significant difference in disinfection efficiency of the disinfectants between the different treated batches, despite the differences in initial microbial load as well as microbial growth during storage (Figure 4).

3.4. Evaluation of water sanitizers to maintain the wash water quality

Washing sugar snaps for up to 3 min had only minimal influence on the physicochemical water quality: turbidity increased from 0.41 ± 0.05 to 1.16 ± 0.71 NTU and absorbance at UV 254 nm (0.45 µm filtered) from 0.020 ± 0.003 to 0.047 ± 0.015. The pH value of the washing solutions did not change significantly after 3 min washing, the free chlorine concentration diminished 1.46 ± 0.08 mg/L when adding 20 mg/L free chlorine and no significant changes were observed when washing with 200 mg/L free chlorine for 3 min. The initial microbial load of the used tap water was 3.6 ± 1.0 log CFU/100 mL APC and 0.5 ± 0.6 log CFU/100

mL Y&M. The degree of microbial contamination transferred from the sugar snaps to the water during washing in water was independent of both washing time and experimental batch.

The washing time had no significant influence on the water disinfectant efficiency to lower the wash water contamination in any of the washing setups, so these values were pooled to increase sample sizes for statistical analysis. On the other hand, the disinfectant concentration had a significant influence on the water disinfectant efficiency of APC and Y&M for ABAV and FVS, although for chlorine (20 to 200 mg/L) and lactic acid ($0.32 - 1.6$ %) this was not the case (Figure 5). The APC wash water contamination (5.2 ± 0.6 log CFU/100 mL) was significantly reduced by chlorine 20 to 200 mg/L (to 1.4 ± 0.6 log CFU/100 mL), ABAV 0.5 to 1.5 % (to 2.7 ± 0.8 log CFU/100 mL), FVS 0.5 % (to 2.7 ± 0.7 log CFU/100 mL) and lactic acid 0.8 to 1.6% (to 3.4 ± 0.8 log CFU/100 mL), whereas the Y&M wash water contamination (3.4 ± 0.6 log CFU/100 mL) was only reduced significantly by chlorine 20 to 200 mg/L (to 1.4 ± 0.5 log CFU/100 mL). Y&M were more resistant to water disinfection with organic acids than APC. For chlorine, both Y&M and APC were reduced to similar numbers and one could argue that the remaining microorganisms were mostly Y&M.

Lactic acid in the range 0.32 to 1.6% reduced APC in the water and on the sugar snaps with on average 1.3 ± 0.6 and 1.3 ± 0.5 log respectively and Y&M with on average 0.1 ± 0.4 and 0.6 ± 0.4 respectively. For the commercial sanitizers based on organic acids, ABAV and FVS, the same pattern was observed, i.e. no significant difference between disinfection efficiency of sugar snaps and water disinfection efficiency, which contrasts the much better performance of free chlorine to inactivate microorganisms in the suspended state (Wilcoxon signed rank test; $p > 0.05$ for lactic acid, FVS, and ABAV, $p < 10E-5$ for chlorine).

4. DISCUSSION

14

Visual microbial spoilage was the limiting factor of shelf-life. Nonetheless, APC and Y&M numbers were ineffective to indicate this visual microbial spoilage, as a large variation in microbial counts of the sugar snaps was found. The heterogeneity among microbial contamination of individual sugar snaps, and the scarcity of sugar snaps that actually show signs of microbial spoilage makes it unlikely to pinpoint excessive microbial growth through sampling. Also, APC and Y&M generally do not provide information about individual species and the growth of specific spoilage microorganisms might be masked by these broad-spectrum microbial analyses (Gram et al., 2002). Nonetheless, identifying and measuring the specific spoilage microorganisms would not solve the problem of variation in microbial counts within a batch as observed in this study. Although some studies observed that the level of total microbial counts or specific spoilage microorganisms in fresh produce were not related to the product quality and shelf-life (Allende et al., 2008; Bennik et al., 1998; Gimenez et al., 2003; Ragaert et al., 2007; Zagory, 1999), others have shown a good correlation between sensorial shelf-life and microbial numbers, such as Chen et al. (2010) (correlation with aerobic mesophilic and psychrotrophic bacteria and yeasts and molds on fresh-cut asparagus lettuce) and Jacxsens et al. (2003) (correlation with yeasts and lactic acid bacteria on mixed bell peppers and grated celeriac). The type of microbial spoilage and sensorial quality deterioration depends on the type of fresh produce (Jacxsens et al., 2003). It is plausible that increased understanding of the specific spoilage microorganisms, spoilage mechanisms, and produced metabolites in a certain type of fresh produce, will enable a better prediction of shelf-life through microbial measurements, not considering the microbial variability issues observed in this study.

Characteristics explaining physical damage and the physiological status of the sugar snaps, i.e. the maturity of the sugar snaps and the integrity of the pod at the calyx end, were more

predictive towards the visual shelf-life of the sugar snaps. This illustrates that harvesting at the right stage of maturity, avoiding damage during harvesting and more thorough visual selection before processing could lead to an end product with longer shelf-life. Fungal spoilage usually originates from latent infections established in the field or wound infections during harvesting and handling (Terry and Joyce, 2004). *Pseudomonas* spp. and *Erwinia* spp. that colonize plant surfaces adhere preferentially in the natural depression of stomata or in the intercellular junction, or cracks or crevices formed through damage, after which diverse biofilms can arise, composed of gram-negative and gram-positive bacteria, yeasts and filamentous fungi (Carmichael et al., 1999). The weakening natural defense mechanisms of overmature or mechanically damaged sugar snaps and the loss of structural integrity at the calyx ends, potentially leading to increased solute leakage, improves the growth conditions of phytopathogens (Elghaouth et al., 1992; Nunes et al., 2010). Regardless the observed microbial related issues of overmature sugar snaps, sugar snaps should always be harvested before physiological maturity is achieved. Otherwise, excessive sugar to starch conversion results in loss of sweetness and crispness with advancing maturity, becoming tougher and fibrous (Basterrechea and Hicks, 1991; Sams, 1999).

Chlorine was confirmed to be an efficient, fast acting water disinfectant against vegetative bacteria as observed in previous studies (Lee et al., 2010; Luo et al., 2011; Van Haute et al., 2013). Chlorine also effectively removed yeasts and molds from the wash water, but did not significantly enhance the Y&M reduction on the sugar snaps compared to a water wash. Pereira et al. (2013) reported fungi to be more resistant to chlorination in drinking water than bacteria and viruses, but less resistant than *Cryptosporidium* oocysts. Beuchat et al. (1998) suggested a large abundance of chlorine resistant cell types among fungi. Contrary to its efficiency to remove suspended, vegetative microorganisms, chlorine is much less efficient as

16

fresh produce decontaminant, a behavior shared among the chemical water disinfectants chlorine, chlorine dioxide, and ozone and observed in a myriad of studies. Decontamination processes are compromised by presence of microorganisms in biofilm, attachment to and internalization through surface wounds and stomata, internalization through the plant roots and subsequent migration throughout the plant, and increasing surface roughness of the produce at microscopic level (Gomez-Lopez et al., 2008; Huang et al., 2006; Jahid and Ha, 2012; Luo et al., 2011; Takeuchi and Frank, 2001; Wang et al., 2009). Although gaseous chlorine, chlorine dioxide, hydrogen peroxide and ozone have a higher diffusion capacity than when dissolved in water and have a higher potential for decontaminating injured and other hard to reach produce surfaces, gaseous disinfectants do not solve the problems of microbial internalization in fresh produce (Gomez-Lopez et al., 2008). Han et al. (2001) observed an increased reduction of spot inoculated *Listeria monocytogenes* of 3 log reduction when applying chlorine dioxide as gas treatment (3 mg/L, 10 min, 20°C) compared to an aqueous chlorine dioxide treatment (3 mg/L, 10 min, 20°C) of both uninjured and injured green peppers. However, Hadjok et al. (2008), who used vacuum infiltration in order to achieve internalization of inoculated *Salmonella* Montevideo in fresh-cut iceberg lettuce, observed that gas exposure of the produce to 1.5% H_2O_2 at 50°C resulted in 2 log reduction on the lettuce, whereas only 0.5 log of the internalized *Salmonella* Montevideo were inactivated.

The behavior of weak organic acids is fundamentally different from chemical oxidants such as chlorine as weak organic acids are not compromised as severely when inactivation of microorganisms is needed in the presence of organics in the water or food matrix, or exopolymeric substances in biofilms. Where chlorine is decomposed through reaction with organic matter, the loss of the 'active substance' of weak organic acids is synonymous with deprotonation, and buffer capacity in the vicinity of the produce surface, as well as alkalinity

17

of the water (both inorganic and organic such as from anions of organic acids with pKa $\geq$ 4) could theoretically pose a disinfection barrier (Hemond, 1990). However, the alkalinity did not change significantly during the 3 hour washing trials in the packaging company. Also, during washing of fresh-cut radicchio, sugar loaf, and endive, a process which generates a higher converged COD (295 ± 8 mg O_2/L), the alkalinity was stable throughout the 135 min washing process at on average 6.38 ± 0.12 mmole/L (own data not published). Unless considerable amounts of buffering substances are introduced during washing, efficiency of organic acids will not be severely influenced by the water matrix during produce washing operations. However, weak organic acids in general are inefficient water disinfectants, and the results in this study show that, given the contact times used, the efficacy to inactivate microorganisms in suspended state is not better than the reduction of microorganisms on sugar snaps surfaces. Virto et al. (2006) modeled the inactivation of *Listeria monocytogenes* and *Escherichia coli* in function of concentration of citric or lactic acid, temperature and contact time. To achieve a 3 log reduction in sterile distilled water at 5°C and with 1.6 % lactic acid (the most severe lactic acid settings applied in this study) would take 25 min and 35 min of contact time for *Escherichia coli* and *Listeria monocytogenes* respectively. In this study, the short contact times applied (range 30 to 180 s), in combination with the experimental variability, masked the water disinfection kinetics of lactic acid. For comparison of lactic acid and free chlorine as water disinfectants, according to Chick-Watson kinetics, it would take 10 s for 1 mg/L free chlorine at pH 6.5 in oxidant demand free buffer to reduce *Escherichia coli* O157 by 3 log (Van Haute et al., 2013). Lactic acid, FVS, and ABAV failed at effectively reducing Y&M, both on the produce and in the wash water. The resistance of spoilage fungi to organic acids is related to the membrane ATPase activity and pH homeostase mechanisms such as acid anion efflux pumps (Brul and Coote, 1999; Smits and Brul, 2005).

Within the studied parameter ranges, contact time had little influence on the decontamination effectiveness of the sugar snaps decontamination treatments. The same was observed for concentration, except for ABAV and lactic acid which showed increased (yet not significant) reduction of APC with increased concentration. The decontamination behavior in function of time and concentration and observed in numerous studies can be described as following: the microbial load can initially be reduced quite effectively with limited exposure (concentration x contact time), after which increased exposure is less successful in achieving further microbial reduction (Akbas and Olmez, 2007b; Ayhan et al., 1998; Beuchat et al., 1998; Chen and Zhu, 2011; Mahmoud et al., 2008; Olmez and Akbas, 2009). This again can be explained by the state/location of the microorganisms on fresh produce, comprising of microorganisms that are easily, hard, or virtually impossible to inactivate with water disinfectants. Easily removable microorganisms that are vulnerable against the respective disinfectant require relatively little exposure, whereas those which reside in thick biofilms and stomata, require a much higher exposure. The severity of the exposure might be limited by produce damage or engineering issues such as long duration of produce washing steps, or might be virtually futile in the case of internalized microorganisms. The lack of influence of concentration on decontamination efficiency observed in this study can be explained by i) working in a concentration range in which all concentrations inactivated the easily reachable microorganisms, ii) the lack of disinfection efficiency of a certain disinfectant to remove hard to reach microorganisms, iii) the masking of the possible influence of concentration on decontamination efficiency of difficult to remove microorganisms due to the high variability in microbial counts, iv) the presence of recalcitrant internalized microorganisms. The lack of influence of contact time on decontamination efficiency could be explained by i) working in a too small range of contact times to observe differences, ii) decontamination kinetics of

19

disinfectants (suppose that contact times > 30 s would result in no further significant inactivation), or iii) interference of high variability in microbial counts. Some studies (Akbas and Olmez, 2007b; Ayhan et al., 1998; Beuchat et al., 1998; Olmez and Akbas, 2009) show a more severe limitation of further fresh produce decontamination (and as such less influence of concentration and contact time beyond the initial effective decontamination stage) than others (Chen and Zhu, 2011; Mahmoud et al., 2008) in which further increase in exposure resulted in a more successful further microbial reduction. Different inactivation behavior can be due to several causes: i) fresh produce type and whole *VS* fresh-cut produce, ii) inoculation method or naturally present microflora, iii) the microorganism type, iv) (related to i, ii, and iii) the relative abundance of easily reachable, hard to reach, and infiltrated microorganisms, v) characteristics of the disinfectant (inherent disinfection potential and disinfection kinetics, liquid or gas form), and vi) the applied experimental conditions and execution such as the created turbulence during the washing process.

Except for treatment with lactic acid 0.8 % or chlorine 200 mg/L, gained reductions of the other treatments compared to the untreated sugar snaps were lost in less than 10 days of storage. Microbial regrowth can potentially occur quickly after decontamination due to reduction of competition (Delaquis et al., 1999; Gomez-Lopez et al., 2008; Ragaert et al., 2007). In this study, free chlorine, lactic acid, and ABAV were more effective than a water wash for reduction of APC but not of Y&M on the sugar snaps. Comparison of organic acids and chlorine as fresh produce decontaminants to reduce spoilage microorganisms has also been studied on rocket leaves (Martinez-Sanchez et al., 2006), fresh-cut iceberg lettuce (Akbas and Olmez, 2007a; Allende et al., 2008), fresh-cut escarole (Allende et al., 2008) and fresh-cut cilantro (Allende et al., 2009). Based on those studies, there is no clear, discernible pattern as to whether Y&M are less efficiently removed from fresh produce than mesophilic

or psychrotrophic counts, whether lactic acid or citric acid are more/less efficient than chlorine to remove fungal or bacterial microorganisms from fresh produce, and whether these disinfectants improve the shelf-life of the produce.

The consequences of slow water disinfection kinetics by organic acids, as confirmed in this study, are that organic acids cannot be used to control cross-contamination, which Lopez-Galvez et al. (2009) demonstrated for *Escherichia coli* transfer from inoculated to non-inoculated fresh-cut iceberg lettuce during washing with 2 % Purac or 0.5 % Citrox®. Therefore, it seems organic acids are not suitable for washing applications of fresh produce, although there might be potential for their use in decontamination applications through spraying or electrostatic spraying on fresh produce (Ganesh et al., 2010, 2012), as such bypassing the low water disinfection efficiency by using a method without water immersion. This especially has potential when applied as a warm/hot spray, as research on *Escherichia coli* and *Listeria monocytogenes* suspended in water (4 °C *VS* 20 °C *VS* 40 °C) as well as *Escherichia coli* O157:H7 inoculated on baby spinach (22 °C *VS* 40 °C) has shown that the disinfection efficiency of lactic acid is significantly enhanced by increased temperature (Huang and Chen, 2011; Virto et al., 2006).

Washing of whole produce such as sugar snaps, introduces exudates in the wash water (most probably from wounded surfaces) to a much lesser extent than washing of fresh-cut produce. As such, the transfer of organic materials depends in greater part on foreign organics and particles present on the sugar snaps. The converged COD values (30 ± 5 mg O_2/L) in this study were low compared to the converged COD measured in two fresh-cut leafy vegetable companies by Van Haute et al. (2013), COD 465 ± 2 and 1405 ± 57 mg O_2/L. Therefore, considering the high microbial build-up during washing, the inability of the tested water

sanitizers to prolong the shelf-life, the absence of detrimental effects of chlorine on the sensorial quality of the sugar snaps, the high performance of free chlorine as a water disinfectant, and the low physicochemical load of the sugar snaps wash water which would minimize the disinfection by-products generation, maintaining a free chlorine residual seems to be a suitable strategy to avoid cross-contamination of vegetative bacteria and fungi in the washing process of sugar snaps.

ACKNOWLEDGEMENTS

REFERENCES

Akbas, M.Y., Olmez, H., 2007a. Effectiveness of organic acid, ozonated water and chlorine dippings on microbial reduction and storage quality of fresh-cut iceberg lettuce. Journal of the Science of Food and Agriculture 87, 2609-2616.

Akbas, M.Y., Olmez, H., 2007b. Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatments with organic acids. Letters in Applied Microbiology 44, 619-624.

Allende, A., McEvoy, J., Tao, Y., Luo, Y.G., 2009. Antimicrobial effect of acidified sodium chlorite, sodium chlorite, sodium hypochlorite, and citric acid on *Escherichia coli* O157:H7 and natural microflora of fresh-cut cilantro. Food Control 20, 230-234.

Allende, A., Selma, M.V., Lopez-Galvez, F., Villaescusa, R., Gil, M.I., 2008. Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce. Postharvest Biology and Technology 49, 155-163.

Ayhan, Z., Chism, G.W., Richter, E.R., 1998. The shelf-life of minimally processed fresh cut melons. Journal of Food Quality 21, 29-40.

Basterrechea, M., Hicks, J.R., 1991. Effect of maturity on carbohydrate changes in sugar snap pea pods during storage. Scientia Horticulturae 48, 1-8.

Bennik, M.H.J., Vorstman, W., Smid, E.J., Gorris, L.G.M., 1998. The influence of oxygen and carbon dioxide on the growth of prevalent Enterobacteriaceae and *Pseudomonas* species isolated from fresh and controlled-atmosphere-stored vegetables. Food Microbiology 15, 459-469.

Beuchat, L.R., Nail, B.V., Adler, B.B., Clavero, M.R.S., 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. Journal of Food Protection 61, 1305-1311.

Brandl, M.T., Sundin, G.W., 2013. Focus on food safety: human pathogens on plants. Phytopathology 103-4, 304-305.

Brul, S., Coote, P., 1999. Preservative agents in foods - mode of action and microbial resistance mechanisms. International Journal of Food Microbiology 50, 1-17.

Brummell, D.A., 2006. Cell wall disassembly in ripening fruit. Functional Plant Biology 33, 103-119.

Capozzi, V., Fiocco, D., Amodio, M.L., Gallone, A., Spano, G., 2009. Bacterial stressors in minimally processed food. International Journal of Molecular Sciences 10, 3076-3105.

Carmichael, I., Harper, I.S., Coventry, M.J., Taylor, P.W.J., Wan, J., Hickey, M.W., 1999. Bacterial colonization and biofilm development on minimally processed vegetables. Journal of Applied Microbiology 85, 45S-51S.

Chen, Z., Zhu, C.H., Zhang, Y., Niu, D.B., Du, J.H., 2010. Effects of aqueous chlorine dioxide treatment on enzymatic browning and shelf-life of fresh-cut asparagus lettuce (*Lactuca sativa* L.). Postharvest Biology and Technology 58, 232-238.

Chen, Z., Zhu, C., 2011. Modelling inactivation by aqueous chlorine dioxide of *Dothiorella gregaria Sacc.* and *Fusarium tricinctum* (Corda) *Sacc.* spores inoculated on fresh chestnut kernel. Lett Appl Microbiol 52, 676-684.

Critzer, F.J., Doyle, M.P., 2010. Microbial ecology of foodborne pathogens associated with produce. Current Opinion in Biotechnology 21, 125-130.

Delaquis, P.J., Stewart, S., Toivonen, P.M.A., Moyls, A.L., 1999. Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. Food Research International 32, 7-14.

Eaton, A.D, Clesceri, L.S, Rice, E.W, Greenberg, A.E, 2005. Standard methods for the examination of water and wastewater, 21st ed. American Public Health Association, Washington, DC.

Elghaouth, A., Arul, J., Asselin, A., Benhamou, N., 1992. Antifungal activity of chitosan on postharvest pathogens – induction of morphological and cytological alterations in *Rhizopus-Stolonifer*. Mycological Research 96, 769-779.

FDA, 2001. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Evaluation and definition of potentially hazardous foods. Chapter 7. Comparison of NSF and ABA protocols to determine whether a food requires time/temperature control for safety. Available:http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm094156.htm.

Ganesh, V., Hettiarachchy, N.S., Griffis, C.L., Martin, E.M., Ricke, S.C., 2012. Electrostatic spraying of food-grade organic and inorganic Acids and plant extracts to decontaminate *Escherichia coli* O157:H7 on spinach and iceberg lettuce. Journal of Food Science 77, M391-M396.

Ganesh, V., Hettiarachchy, N.S., Ravichandran, M., Johnson, M.G., Griffis, C.L., Martin, E.M., Meullenet, J.F., Ricke, S.C., 2010. Electrostatic sprays of food-grade acids and plant extracts are more effective than conventional sprays in decontaminating *Salmonella* Typhimurium on spinach. Journal of Food Science 75, M574-M579.

Gimenez, M., Olarte, C., Sanz, S., Lomas, C., Echavarri, J.F., Ayala, F., 2003. Relation between spoilage and microbiological quality in minimally processed artichoke packaged with different films. Food Microbiology 20, 231-242.

Gomez-Lopez, V.M., Ragaert, P., Debevere, J., Devlieghere, F., 2008. Decontamination methods to prolong the shelf-life of minimally processed vegetables, state-of-the-art. Critical Reviews in Food Science and Nutrition 48, 487-495.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., Givskov, M., 2002. Food spoilage - interactions between food spoilage bacteria. International Journal of Food Microbiology 78, 79-97.

25

Hadjok, C., Mittal, G.S., Warriner, K., 2008. Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. Journal of Applied Microbiology 104, 1014-1024.

Han, Y., Linton, R.H., Nielsen, S.S., Nelson, P.E., 2001. Reduction of *Listeria monocytogenes* on green peppers (*Capsicum annuum* L.) by gaseous and aqueous chlorine dioxide and water washing and its growth at 7 degrees C. Journal of Food Protection 64, 1730-1738.

Hemond, H.F., 1990. Acid neutralizing capacity, alkalinity, and acid-base status of natural-waters containing organic acids. Environmental Science & Technology 24, 1486-1489.

Huang, T.S., Xu, C.L., Walker, K., West, P., Zhang, S.Q., Weese, J., 2006. Decontamination efficacy of combined chlorine dioxide with ultrasonication on apples and lettuce. Journal of Food Science 71, M134-M139.

Huang, Y.X., Chen, H.Q., 2011. Effect of organic acids, hydrogen peroxide and mild heat on inactivation of *Escherichia coli* O157:H7 on baby spinach. Food Control 22, 1178-1183.

Humphrey, J., McCulloch, N., Ota, M., 2004. The impact of European market changes on employment in the Kenyan horticulture sector. Journal of International Development 16, 63-80.

Insulander, M., Svenungsson, B., Lebbad, M., Karlsson, L., de Jong, B., 2010. A foodborne outbreak of *Cyclospora* infection in Stockholm, Sweden. Foodborne Pathogens and Disease 7, 1585-1587.

Jacxsens, L., Devlieghere, F., Ragaert, P., Vanneste, E., Debevere, J., 2003. Relation between microbiological quality, metabolite production and sensory quality of equilibrium modified atmosphere packaged fresh-cut produce. International Journal of Food Microbiology 83, 263-280.

Jahid, I.K., Ha, S.D., 2012. A review of microbial biofilms of produce: future challenge to food safety. Food Science and Biotechnology 21, 299-316.

Johnston, M.A., Harrison, M.A., Morrow, R.A., 2009. Microbial antagonists of *Escherichia coli* O157:H7 on fresh-cut lettuce and spinach. Journal of Food Protection 72, 1569-1575.

Klaiber, R.G., Baur, S., Wolf, G., Hammes, W.P., Carle, R., 2005. Quality of minimally processed carrots as affected by warm water washing and chlorination. Innovative Food Science & Emerging Technologies 6, 351-362.

Lee, E.S., Yoon, T.H., Lee, M.Y., Han, S.H., Ka, J.O., 2010. Inactivation of environmental mycobacteria by free chlorine and UV. Water Research 44, 1329-1334.

Liao, C.S., Fett, W.F., 2001. Analysis of native microflora and selection of strains antagonistic to human pathogens on fresh produce. Journal of Food Protection 64, 1110-1115.

Lofdahl, M., Ivarsson, S., Andersson, S., Langmark, J., Plym-Forshell, L., 2009. An outbreak of *Shigella Dysenteriae* in Sweden, May-June 2009, with sugar snaps as the suspected source. Eurosurveillance 14, 5-7.

Lopez-Galvez, F., Allende, A., Selma, M.V., Gil, M.I., 2009. Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. International Journal of Food Microbiology 133, 167-171.

Luo, Y.G., Nou, X.W., Yang, Y., Alegre, I., Turner, E., Feng, H., Abadias, M., Conway, W., 2011. Determination of free chlorine concentrations needed to prevent *Escherichia coli* O157:H7 cross-contamination during fresh-cut produce wash. Journal of Food Protection 74, 352-358.

Mahmoud, B.S.M., Vaidya, N.A., Corvalan, C.M., Linton, R.H., 2008. Inactivation kinetics of inoculated *Escherichia coli* O157 : H7, *Listeria monocytogenes* and *Salmonella* Poona on whole cantaloupe by chlorine dioxide gas. Food Microbiology 25, 857-865.

Martinez-Sanchez, A., Allende, A., Bennett, R.N., Ferreres, F., Gil, M.I., 2006. Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. Postharvest Biology and Technology 42, 86-97.

Messiaen, C.-M., Seif, A.A., Jarso, M., Keneni, G., 2004. Pisum Sativum L. In: Grubben, G.J.H., Denton, O.A. (Eds.), Plant resources of tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands/Backhuys Publishers, Leiden, Netherlands/ CTA, Wageningen, Netherlands, pp. 419-425.

Nunes, C., Duarte, A., Manso, T., Weiland, C., Garcia, J.M., Cayuela, J.A., Yousfi, K., Martinez, M.C., Salazar, M., 2010. Relationship between postharvest diseases resistance and mineral composition of citrus fruit. Vi International Symposium on Mineral Nutrition of Fruit Crops 868, 417-422.

Olmez, H., Akbas, M.Y., 2009. Optimization of ozone treatment of fresh-cut green leaf lettuce. Journal of Food Engineering 90, 487-494.

Pereira, V.J., Marques, R., Marques, M., Benoliel, M.J., Crespo, M.T.B., 2013. Free chlorine inactivation of fungi in drinking water sources. Water Research 47, 517-523.

Ragaert, P., Devlieghere, F., Debevere, J., 2007. Role of microbiological and physiological spoilage mechanisms during storage of minimally processed vegetables. Postharvest Biology and Technology 44, 185-194.

Ryser, E.T., Hao, J., Yan, Z., 2009. Internalization of pathogens in produce. In: Fan, X., Niemira, B.A., Doona, C.J., Feeherry, F.E., Gravani, R.B (Eds.), Microbial safety of fresh produce. Wiley-Blackwell, USA, IA, pp. 55-82.

Sams, C.E., 1999. Preharvest factors affecting postharvest texture. Postharvest Biology and Technology 15, 249-254.

Shi, X., Wu, Z., Namvar, A., Kostrzynska, M., Dunfield, K., Warriner, K., 2009. Microbial population profiles of the microflora associated with pre- and postharvest tomatoes contaminated with *Salmonella* Typhimurium or *Salmonella* Montevideo. Journal of Applied Microbiology 107, 329-338.

Smits, G.J., Brul, S., 2005. Stress tolerance in fungi - to kill a spoilage yeast. Current Opinion in Biotechnology 16, 225-230.

Takeuchi, K., Frank, J.F., 2001. Quantitative determination of the role of lettuce leaf structures in protecting *Escherichia coli* O157 : H7 from chlorine disinfection. Journal of Food Protection 64, 147-151.

Teplitski, M., Warriner, K., Bartz, J., Schneider, K.R., 2011. Untangling metabolic and communication networks: interactions of enterics with phytobacteria and their implications in produce safety. Trends in Microbiology 19, 121-127.

Terry, L.A., Joyce, D.C., 2004. Elicitors of induced disease resistance in postharvest horticultural crops: a brief review. Postharvest Biology and Technology 32, 1-13.

Tournas, V.H., 2005. Spoilage of vegetable crops by bacteria and fungi and related health hazards. Critical Reviews in Microbiology 31, 33-44.

Van Haute, S., Sampers, I., Holvoet, K., Uyttendaele, M., 2013. Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. Applied and Environmental Microbiology 79, 2850-2861.

Virto, R., Sanz, D., Alvarez, I., Condon, S., Raso, J., 2006. Application of the Weibull model to describe inactivation of *Listeria monocytogenes* and *Escherichia coli* by citric and lactic acid at different temperatures. Journal of the Science of Food and Agriculture 86, 865-870.

Walker, R., Powell, A.A., Seddon, B., 1998. *Bacillus* isolates from the spermosphere of peas and dwarf French beans with antifungal activity against *Botrytis cinerea* and *Pythium* species. Journal of Applied Microbiology 84, 791-801.

Wang, H., Feng, H., Liang, W., Luo, Y., Malyarchuk, V., 2009. Effect of surface roughness on retention and removal of *Escherichia coli* O157:H7 on surfaces of selected fruits. Journal of Food Science 74, E8-E15.

Zagory, D., 1999. Effects of post-processing handling and packaging on microbial populations. Postharvest Biology and Technology 15, 313-321.

FIGURE CAPTIONS

Figure 1. Microbial contamination in the washing bath during the washing of sugar snaps (APC (♦), Y&M (■), LAB (●)). The data points at t = -25 min show the microbial quality of the used tap water. Error bars denote standard deviation (n=3).

Figure 2. COD (♦) and turbidity (■) in the washing bath during sugar snaps washing. The data points at t = -25 min show the COD/turbidity of the used tap water. Error bars denote standard deviation (n=3).

Figure 3. Microbial load of the untreated sugar snaps in function of experimental batch and storage time. Numbers in the graphs indicate the batch on which visual microbial decay was observed at that storage time. Error bars denote standard deviation (n=3).

Figure 4. Microbial load in function of storage time for both samples that showed visual microbial decay during storage (APC (♦), Y&M (■)) and those that did not (APC (◊), Y&M (□)). Batches consisted of 36 to 48 samples, error bars denote standard deviation.

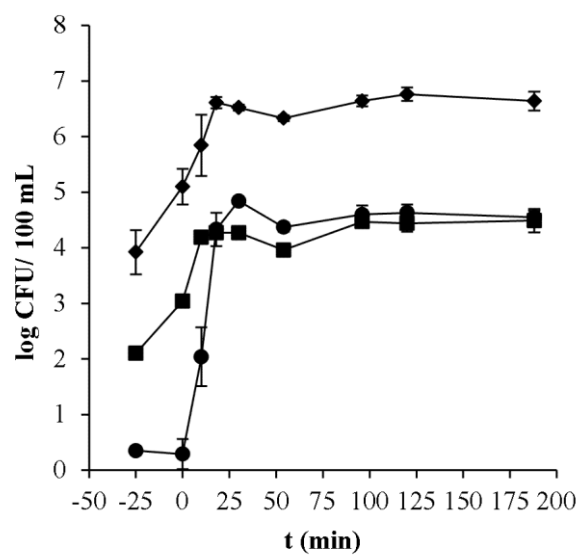Figure 5. Microbial wash water contamination during sugar snaps decontamination experiments. Error bars denote standard deviation (n=9).
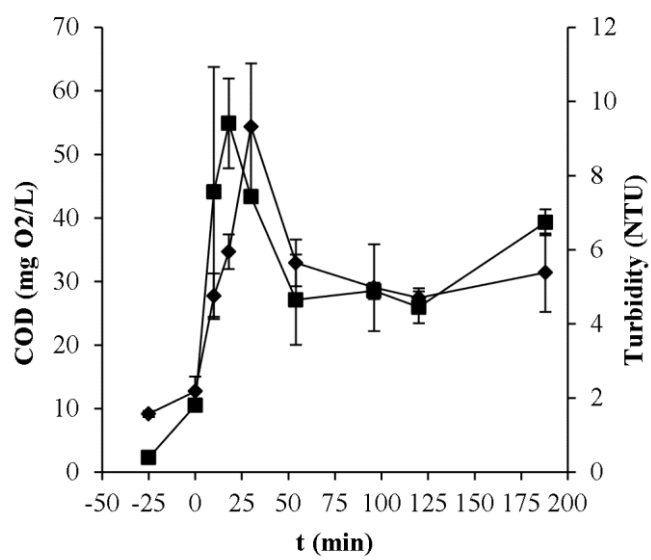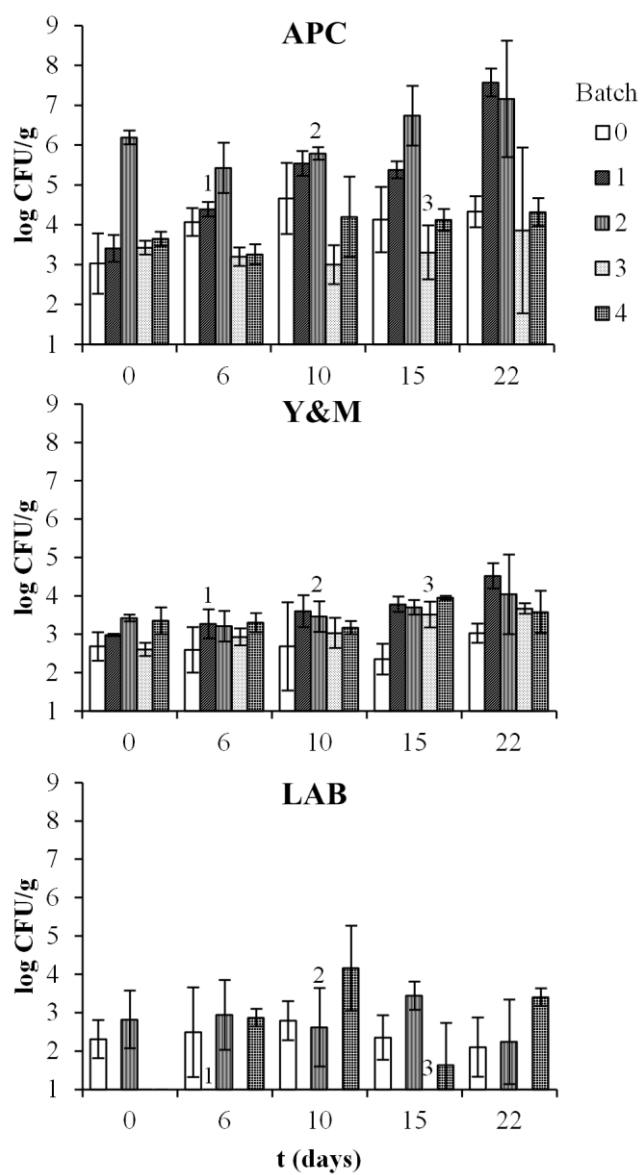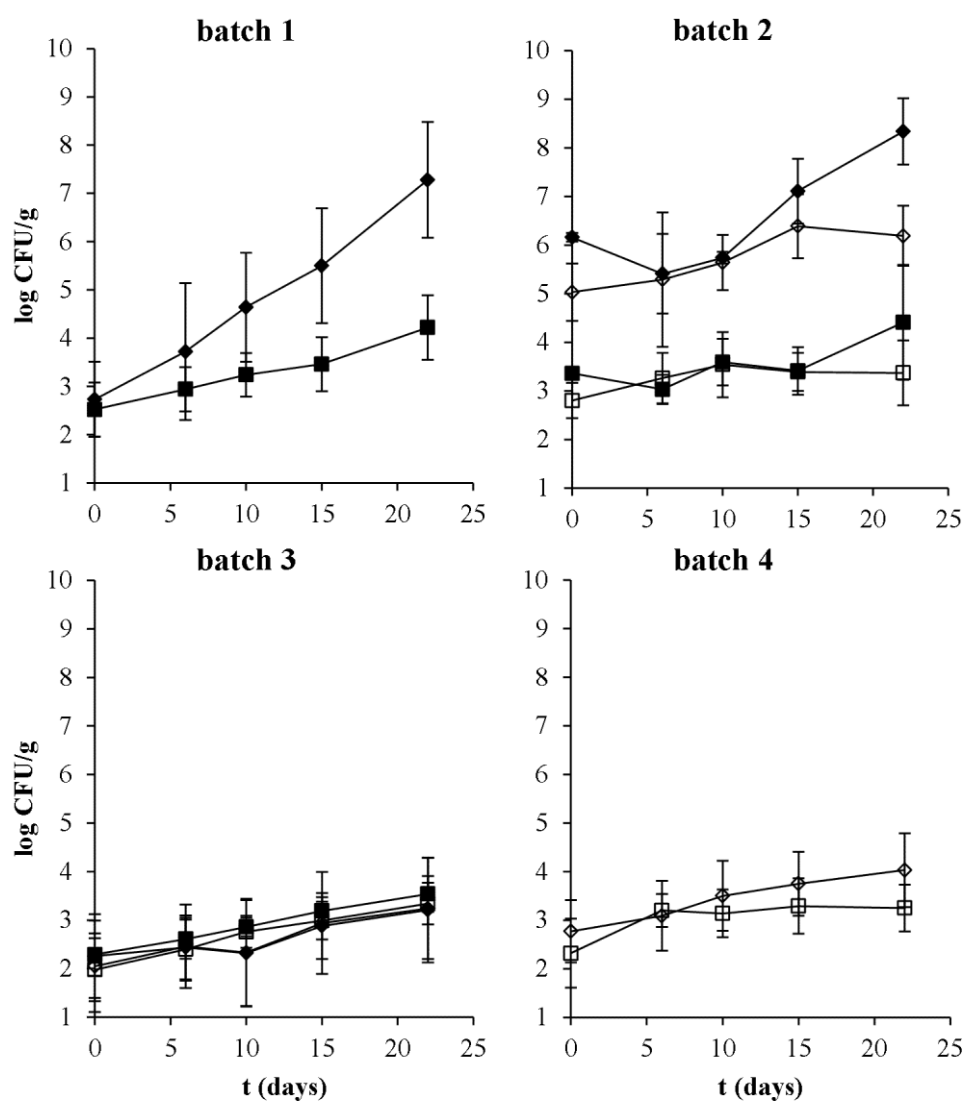
Fig. 1

Fig. 2

Fig. 3

Fig. 4
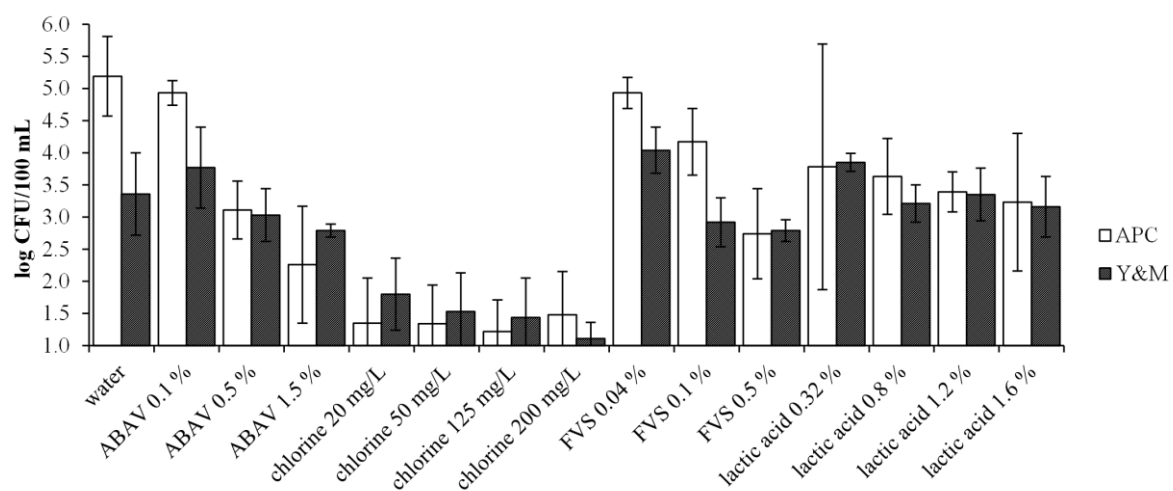
Fig. 5

Table 1. The experimental concentration-time settings used in the decontamination experiments (n=3).

Disinfectant	Used settings	Settings that did not impact the sensorial quality	Rinsing
Chlorine	20,50,125,200 mg/L 30,60,180 s pH 6.5 (HCl)	all were usable	not necessary, no impact on product
Acetic acid	0.8,1.2,1.6 % 30,60 s	all settings caused defects, also generation of noxious vapors from the washing bath	rinsing removed off-odors but not the other issues
Lactic acid	0.32,0.8,1.2,1.6 % 30,60,180 s	0.32 % (30-180 s) 0.8 % (30-60 s) 1.2-1.6 % (30 s)	necessary
ABAV	0.1,0.5,1.5 % 30, 60, 180 s	0.1 % (30-180 s) 0.5 % (30 s)	were never rinsed[a]
FVS	0.04,0.1,0.5 % 30, 60, 180 s	0.04-0.1 % (30-180 s) 0.5 % (30 s)	were always rinsed[a]
Water	30,60,180 s		

[a] The recommendations of the manufacturer were followed.

Table 2. Comparison of visual characteristics of different batches untreated sugar snaps (n=3).

Batch	Maturity (seed size)	Damage to calyx end	Other mechanical damage	Visual microbial decay (days storage)
0	I-M[a]	+[b]	++	/[c]
1	OM	+++	+	6 (3/3)[d]
2	M	+	++	10 (1/3)
3	M	++	++	15 (1/3), 22 (2/3)
4	I	+	+++	/

[a]I : immature, M : mature, OM : overmature, [b]Number of '+' expresses relative severity of characteristic, [c]No visual microbial decay observed in 22 days of storage, [d]fraction of samples that showed decay.

Table 3. Microbial log reduction on sugar snaps during storage.

		Storage time (day)				
		0	6	10	15	22
APC	Untreated (n=12)	0.0±0.2	0.0±0.3	0.0±0.5	0.0±0.5	0.0±0.8
	Water (n=12)	0.5±0.7	-0.4±0.7	0.3±0.5	0.0±0.8	0.5±0.9
	ABAV 0.1 % (n=9)	0.6±0.8	-0.2±1.0	0.6±0.7	-0.2±1.0	-0.3±1.2
	ABAV 0.5 % (n=3)	1.6±0.1 [ABa]	0.4±0.2	1.0±0.9	0.4±0.3	2.0±0.6 [AB]
	Chlorine 20 mg/L (n=9)	1.2±0.5 [A]	0.4±0.8	1.0±0.4 [A]	1.0±0.4 [A]	0.3±0.9
	Chlorine 50 mg/L (n=9)	1.1±0.6 [A]	0.0±0.5	0.4±0.8	0.1±1.0	0.1±1.0
	Chlorine 125 mg/L (n=9)	1.1±0.3 [A]	0.0±0.2	0.4±1.1	0.5±0.2	1.1±0.8
	Chlorine 200 mg/L (n=9)	1.4±0.5 [AB]	0.5±0.8 [B]	0.7±0.7 [A]	0.3±0.8	0.7±1.2
	FVS 0.04 % (n=9)	0.7±0.8	-0.2±0.7	0.4±0.5	0.2±0.5	0.1±0.6
	FVS 0.1 % (n=9)	1.0±0.5 [A]	0.4±0.1	-0.1±0.9	0.4±0.6	1.3±1.2
	FVS 0.5 % (n=3)	0.6±0.8 [A]	1.1±0.4 [AB]	0.7±1.3	0.3±1.2	1.7±1.5 [A]
	Lactic acid 0.32 % (n=9)	0.9±0.4 [A]	0.1±0.7	1.0±0.5 [A]	0.6±0.4	0.6±0.5
	Lactic acid 0.8 % (n=6)	1.2±0.5 [AB]	0.6±1.1 [AB]	1.3±1.1 [AB]	0.1±1.6	0.4±1.1
	Lactic acid 1.2 % (n=3)	1.5±0.4 [AB]	0.2±0.9	0.5±1.6	0.7±0.5	1.4±0.3 [A]
	Lactic acid 1.6 % (n=3)	1.8±0.2 [AB]	0.2±0.3	0.1±0.2	1.2±1.2 [AB]	1.1±0.6
Y&M	Untreated	0.0±0.2	0.0±0.3	0.0±0.3	0.0±0.2	0.0±0.5
	Water	0.6±0.4 [A]	0.0±0.5	0.2±0.3	0.5±0.3	0.6±0.6 [A]
	ABAV 0.1 %	0.8±0.2 [A]	0.1±0.4	-0.2±0.3	0.4±0.7 [A]	0.2±0.5
	ABAV 0.5 %	0.6±0.4	0.1±0.1	0.1±0.1	0.3±0.1	0.4±0.2
	Chlorine 20 mg/L	1.2±0.7 [A]	0.3±0.5	0.6±0.7 [A]	1.1±0.6 [AB]	0.5±0.6 [A]
	Chlorine 50 mg/L	0.8±0.7 [A]	0.2±0.4	0.3±0.5	0.7±0.5 [A]	0.7±0.6 [A]
	Chlorine 125 mg/L	0.9±0.2 [A]	0.3±0.3	0.4±0.6	0.7±0.6 [A]	0.9±0.4 [A]
	Chlorine 200 mg/L	1.0±0.9 [A]	0.7±0.4 [AB]	0.5±0.4 [A]	0.9±0.6 [A]	0.6±0.4 [A]
	FVS 0.04 %	0.8±0.6 [A]	0.0±0.3	-0.4±0.3	0.6±0.1 [A]	0.3±0.2
	FVS 0.1 %	0.3±0.2	0.0±0.3	0.3±0.3	0.4±0.2	0.2±0.5
	FVS 0.5 %	0.2±0.3	0.4±0.3	0.2±0.2	0.3±0.2	-0.2±0.3
	Lactic acid 0.32 %	0.8±0.6 [A]	-0.1±0.4	-0.1±0.3	0.4±0.3	0.0±0.6
	Lactic acid 0.8 %	0.5±0.5	0.1±0.6	0.0±0.5	0.2±0.5	0.3±0.5
	Lactic acid 1.2 %	0.7±0.3 [A]	-0.2±0.4	-0.3±0.9	0.3±0.5	0.4±0.2
	Lactic acid 1.6 %	0.6±0.2	-0.1±0.4	-0.5±0.4	0.1±0.3	0.6±0.4 [A]

[a]for APC and Y&M separately, [A] denotes a significant microbial reduction compared to the untreated samples, [B] denotes a significant microbial reduction compared to a water wash.

Table 4. Overview of sugar snaps samples that showed visual microbial decay in the different batches in function of storage time.

	Batch 1 day				Batch 2 day				Batch 3 day				Batch 4 day			
	6	10	15	22	6	10	15	22	6	10	15	22	6	10	15	22
Untreated	3/3[a]						1/3				1/3	2/3				0/3
Water	3/3							0/3				0/3				0/3
Chlorine	12/12						1/12					0/4				0/8
Lactic acid	12/12							0/12				2/8				0/4
ABAV				/[b]				0/9			3/9	7/9				0/9
FVS				/				0/9			2/9	6/9				0/9

[a]fraction of samples that showed visual microbial decay, [b]was not executed in that batch.

HIGHLIGHTS

- Organic acid based sanitizers and chlorine were used to decontaminate sugar snaps
- Sanitizers failed to extend shelf-life of sugar snaps (visual microbial spoilage)
- Maturity and damage at calyx end of pods predicted visual microbial spoilage
- Low physicochemical and high microbial build-up in water during sugar snaps washing
- Chlorine maintained bacterial and fungal wash water quality, organic acids did not