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FINAL PROJECT REPORT

Project Title
Establishment of operating standards for produce wash systems through the identification of specific metrics and test methods

Project Period
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Objectives
1. *Identification of specific water quality variables suitable as critical parameters and their critical and optimal operational limits for water disinfection under commercial conditions. Monitoring will be performed in commercial facilities covering 98% of the most commonly used disinfection treatments (e.g. chlorine-based sanitizers and peroxyacetic acid) in multiple commodity types representing the best and worse scenarios of process wash water.*
2. *Validation of critical limits of the selected critical parameters. Validation of critical parameters will be performed in lab-scale experiments using artificially inoculated foodborne pathogens.*
3. *Performance of commercial sensors and test kits for the on-line monitoring of the critical parameters that can function adequately because of the accuracy, precision and low range of error.*
4. *Verification of critical and operational limits. Verification of the selected critical and operational limits will be carried out on-line to confirm the performance of the monitoring standards used to control the microbiological quality of the process wash water.*
5. *Elaboration of evidence-based standards for the most common disinfectants based on specific metrics and test methods for microbiological quality of produce wash systems, to meet the immediate need in the process control for produce industry operators.*

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FINAL REPORT

Abstract

Fresh produce wash water disinfection at the industrial scale is essential to reduce the microbiological safety risk for consumers. However, managing this process is a difficult task due to the constantly changing conditions in commercial settings. Ideally, wash water treatment should reduce the risk of microbial cross-contamination between batches of product, without increasing the chemical risk due to the formation and accumulation of disinfection by-products. The results are evidence that the parameters critical for the management of the washing process are specific of each washing system and product type. Reliable and sound methods for the measurement of such factors have been defined. Throughout this project, mainly industrial-scale data but also lab-scale data were obtained to identify and validate water quality variables suitable as critical parameters for the washing of fresh produce. Disinfectant concentration, pH, and organic matter levels are the most critical factors in disinfection systems using chlorine. The absorbance of water at 254 nm (UV254) was identified as a metric that could be used to monitor chlorine demand due to the organic matter content of the produce wash water to make water replenishment decisions. In the case of peroxyacetic acid (PAA), the critical factor to be controlled is the concentration of the disinfectant. Furthermore, the performance of different commercial sensors and test kits for the measurement of the selected parameters was assessed, and the best available methods were identified. An amperometric method (ChloroSense, Palintest) and a DPD-based method (Spectroquant, Merck) were demonstrated to be reliable methods for chlorine measurement in the produce wash water. The amperometric method PAASense (Palintest) is a fast and accurate sensor for the quantification of PAA concentration in industrial wash water. In addition, operational limits for wash water disinfection under industrial conditions were established and verified. In the case of systems using chlorine as a disinfectant, the minimum operational limit in fresh-cut lettuce, cabbage, onion, and baby leaves wash water was set as 20–25, 20–25, 10, and >30 mg/L, respectively. Evidence-based standards that take into account the differences between washing operations, including the disinfectants used and the type of product washed, are proposed.

Background

Safety issues associated with produce wash systems

The fresh produce industry consumes significant volumes of water, mainly in the product washing processes, and high quantities of wastewater are generated daily (Manzocco et al., 2015). This high water demand makes water reuse a necessity. There is a need for methods to preserve the microbiological and chemical quality of the process water, allowing its reuse without compromising food safety (Casani et al., 2005).

In the washing step, effective antimicrobial treatment of wash water is needed to avoid cross-contamination between contaminated and uncontaminated plant material, reducing the risk linked to a higher occurrence of pathogenic microorganisms in the produce (Gombas et al., 2017). Hamilton et al. (2005) and Holvoet et al. (2012) detected fecal contamination indicators in non-disinfected wash water of fresh produce processing plants. It is known that sanitizers present in process wash water have limited efficacy regarding the microbial reductions in produce, while they are effective for the reduction of microbial contamination in wash water (Allende et al., 2008; Pao et al., 2012). The presence of disinfection by-products (DBPs) in the wash water is another safety issue, as the accumulation of these chemical residues in the produce poses a potential safety risk for consumers (EFSA, 2015).

Chlorine and alternative disinfectants

Chlorine, as a produce wash water disinfectant, has some advantages, like its affordability and its demonstrated efficacy at an industrial scale. However, it also has some drawbacks. For example, its employment is linked to the presence of DBPs, its effectiveness is affected by the presence of organic matter, and proper pH control is needed to ensure its antimicrobial efficacy (Suslow, 2001). The DBPs linked with the use of chlorine include, among others, chlorate, trihalomethanes (THMs), and haloacetic acids (HAAs). There is a lack of information on the differences in the formation/accumulation of DBPs during fresh produce washing when using different chlorine derivatives, including sodium hypochlorite, calcium hypochlorite, and chlorine gas. It is known that the use of sodium hypochlorite for the treatment of drinking water leads to higher chlorate levels compared with the use of chlorine gas (Alfredo et al., 2015).

Peroxyacetic acid (PAA) is one of the alternatives to chlorine, and the food industry is the largest user on a global scale (Luukkonen & Pehkonen, 2017). PAA has some positive aspects like its effectiveness in a wide range of pH, its stability in the presence of organic compounds, and no harmful disinfection by-products are formed (Santoro et al., 2007). However, PAA has a higher cost and slower microbial inactivation kinetics than chlorine (Van Haute et al., 2015).

Another alternative disinfectant is chlorine dioxide whose effectiveness is less affected by the pH and the presence of organic matter compared with chlorine (Van Haute et al., 2017). It does not form THMs, but it leads to the presence of chlorite, chlorate, and perchlorate (AHDB, 2016).

Lack of operational standards for specific produce wash systems

The evaluation of the efficacy of washing operations is challenging due to the continuous change in production conditions (Warriner & Namvar, 2013). Usually, different products are manipulated in the same production line without taking into account the requirements of each product (López-Gálvez et al., 2018). There are also variations in product throughput during the working day, in the number of steps (double or single wash), in the equipment design and capacities (e.g., the volume of water and water flow in the washing tanks), the quantity and position of rinsing steps, and the water replenishment policy among others.

In the last decade, there has been scientific work devoted to improving the management of fresh produce washing operations (Azimi et al., 2017; CPS, 2016; Gombas et al., 2017; Zhou et al., 2014). However, there is still a need to establish the critical parameters and their operational limits in different washing systems. Ideal management of wash water treatment seeks the reduction of the microbial risk while avoiding the chemical risk linked to the presence of DBPs (Gil et al., 2009). Together with the antimicrobial efficacy, the formation and accumulation of disinfection DBPs is the primary concern in the washing step of fresh produce (Gil et al., 2016; Shen et al., 2016). For each particular commodity and washing system, an operational range defined by a minimum and a maximum disinfectant level should be established based on the microbial and chemical risk reduction.

The concentration of the disinfectant in the wash water is the most critical parameter to control and guarantee the efficiency of the washing system (López-Gálvez et al., 2018). In the case of the concentration of the disinfectant, the lower operational limit would be defined by the minimum effective concentration that is sufficient to control the microbial quality of the wash water. On the other hand, the higher operational limit would be the maximum concentration of disinfectant that can be applied without creating a safety risk linked to the presence of DBPs. Fresh produce companies should maintain disinfectant concentrations in the wash water between the lower and the higher operational limits. Management of produce wash water sanitation using chlorine-based disinfectants has been extensively studied (Azimi et al., 2017; Chen and Hung, 2016; Gil et al., 2016; Gombas et al., 2017). However, operational limits for chlorine and alternative disinfectants, including peroxyacetic acid and chlorine dioxide, have not been established for specific produce wash systems. Based on lab-scale studies, minimum residual free chlorine (FC) concentrations of 2–10 mg/L for the inactivation of *E. coli* O157:H7 in lettuce and spinach process wash water have been suggested (Luo et al., 2011; Munther and Wu, 2013; Gómez-López et al., 2014; Gombas et al., 2017). Using industrial-scale information, Luo et al. (2018) proposed 10 mg/L as the effective FC concentration for fresh-cut lettuce and cabbage wash water.

Apart from the concentration of disinfectant, each washing operation has other critical parameters. For example, in the case of chlorine, pH has to be controlled, as a suitable pH is crucial for the antimicrobial action of chlorine in the wash water (Suslow, 2001). Generally, the industry sets a higher pH than the one at which the maximum concentration of hypochlorous acid is present. Assuming that the pH regulation is optimal, there are differences depending on the pH regulator that is used (e.g., citric acid, phosphoric acid). The accumulation of organic matter in the wash water is another critical parameter to monitor, not only because of its impact in disinfectant demand and consumption but also due to the formation and accumulation of DBPs. Ideally, the maximum level of organic matter in the water (higher operational limit) should be set for each washing operation, taking into account the amount of DBPs accumulated in the wash water that can be adsorbed by the product.

Performance of commercial sensors and test kits under commercial conditions

Critical parameters for each washing operation must be monitored, and accurate measurement methods must be used. The characteristics of wash water change continuously and the levels of residual disinfectant, pH and organic load are critical parameters to monitor for the optimal performance of wash water disinfection. In many cases, fresh-cut produce companies are not using reliable methods and sound equipment needed for the control of the target parameters. Simple, accurate, rapid, and inexpensive measurement procedures are required.

The concentration of sanitizers in the process wash water is the most critical parameter that should be continuously monitored to ensure the adequate performance of the washing system. Chlorine is usually measured by a quick test kit based on the DPD (N,N-diethyl-p-phenylenediamine) method. However, the accuracy of test kits is poor as they require dilutions that, if they are not performed correctly, introduce some error in the measurement. Furthermore, in some cases, these test kits include subjective interpretation of the result (e.g., comparison of the outcome with color charts by the operator). Due to these problems, it is necessary to select those procedures that are easy to use and accurate to perform the measurements of free and total chlorine. One option is the use of test strips measured by reflectometers as an objective measure that reduces the error introduced by the operators (e.g., non-subjective evaluation of the results). Another option is a new sensor technology based on chronoamperometry that Palintest (Gateshead, UK) has developed in portable instruments for accurate measurements of free and total chlorine. Chronoamperometry involves applying a fixed voltage to an electrode and recording the resulting current over time; the magnitude of the current is proportional to the concentration of chlorine in the test sample. This sensor technology is not affected by solids or sample color.

Regarding PAA, various methods for the measurement of residual concentration have been evaluated in wastewater (cerimetric-iodometric titration, spectrophotometric methods [e.g., DPD], reflectometry, amperometry, and optical biosensors) (Cavallini et al., 2013; Luukkonen & Pehkonen, 2017). On-line sensors including amperometry sensors for monitoring PAA in the produce wash water are very useful, but in many cases, reliable measurements are not achieved as some organic residues such as pesticides or hydrogen peroxide interfere, causing underestimation or overestimation of the levels. Based on the same methodology described for chlorine, Palintest has developed the PAASense as a simple, accurate, rapid and inexpensive measurement procedure for PAA. This new sensor has been evaluated as a precise methodology similar to HPLC measurement as the most specific analytical technique.

In general, two methods should be used, one for monitoring the concentration of the disinfectant and another for the calibration and validation of the performance of the on-line sensors. For this, methods based on reflectometry or spectrophotometry are recommended.

Main goal of the project

The primary goal of the project was to obtain scientific-based evidence for the selection of specific metrics to establish the operational limits necessary for wash water disinfection. Different fresh produce washing systems and disinfection agents were examined. Commercial methods and sensors for the accurate measurement of disinfectant residuals were tested. A user guideline was developed as a manual containing the operational standards of produce wash disinfection systems including chlorine derivatives and PAA.

Research Methods and Results

Research methods

During the two years of the project, most of the experimental studies were performed in washing lines from different fresh produce companies. The collaboration with the industrial partners was essential for the correct performance of the samplings. Preliminary visits to the companies were established for the coordination and the programming of the studies. Samples of water and produce were taken over time in different places of the washing system to obtain microbial and physicochemical data. Other samplings were devoted exclusively to assess the performance of sensors and test kits for the measurement of disinfectant residuals. Also, lab-scale tests on the inactivation of pathogenic microorganisms were performed in controlled conditions to confirm industrial-scale observations.

The facilities of the industrial collaborators, including Fruca, Primaflor, and Florette Iberica, were visited during the project to obtain microbial and physicochemical data at commercial conditions. Additionally, two samplings were performed in the facilities of the company CASI (Cooperativa Agrícola San Isidro, Almería, Spain), one of the major tomato distributors in Spain. A total of ten different washing lines were evaluated, and some of them were sampled more than once, for a total of 21 samplings. The studied products included both cut and whole produce. The cut products comprised leafy greens (different types of baby leaves and fresh-cut lettuce), diced onion, and shredded carrots and red cabbage. The whole products included bell pepper, peeled garlic cloves, and tomato. The disinfectants applied in the studied washing systems included different forms of chlorine (NaClO, $Ca(ClO)_2$, and chlorine gas (Cl_2)), peroxyacetic acid (PAA), and chlorine dioxide (ClO_2). **Table 1** summarizes relevant information about the different samplings performed. The studies were planned with the aim of gathering information on the washing process and adapted to the characteristics of each washing line. The configuration and management of the washing varied widely between companies, and between different lines from the same company in each processing plant. Information on the basic organization of the ten lines assessed during the project is shown in **Table 2**. Water samples were taken periodically from different places of the washing lines (first wash, second wash, and rinse). Physicochemical and microbial characteristics of the water were assessed. The physicochemical parameters studied included pH, temperature, oxidation-reduction potential (ORP), electrical conductivity, the concentration of disinfectant (free and total chlorine, peroxyacetic acid, chlorine dioxide), turbidity, total dissolved solids, absorbance at 254 nm (UV254), chemical oxygen demand (COD) as a measurement of the content of organic matter, and presence of disinfection by-products (DBPs; chlorate and trihalomethanes). The microbial groups measured in all the samplings included total aerobic bacteria (TAB), total coliforms, and *E. coli*. Additionally, *Enterobacteriaceae* were determined in one bell pepper sampling. Free chlorine and total chlorine were measured by the DPD method (APHA, 1998) using the Spectroquant NOVA 60 photometer (Merck, Darmstadt, Germany) and the corresponding test kits. PAA levels were measured by reflectometry using the Reflectoquant system (Merck). Measurements of ClO_2 were carried out by chronoamperometry analysis (Chlordioxense; Palintest). Temperature, ORP, pH, and electrical conductivity were determined using a portable multimeter sensION+ MM150 (Hach, Loveland, Colorado, USA). Turbidity was measured using a turbidimeter Turbiquant 3000 IR (Merck). Total dissolved solids were analyzed using the Standard method 2540 C (APHA, 1998). For UV254 measurement, process wash water was filtered through 0.45-µm syringe nylon filters (Fisherbrand-Fisher Scientific, Waltham, USA) and the absorbance at 254 nm was measured with a UV-VIS spectrophotometer (Jasco V-630, Tokyo, Japan) and using quartz cuvettes with a 1-cm path length (Hellma, Müllheim, Germany). COD was determined by the standard photometric method (APHA, 1998) using a photometer (Spectroquant NOVA 60, Merck). The concentration of chlorates in wash water was assessed in three samples per sampling time using a UPLC-MS as described previously by Gil et al. (2016). Trihalomethanes (THMs) were analyzed by GC-MS as described by Gómez-López et al. (2013), and the areas of the peaks detected by MS were used for quantitation. For THMs, the EPA 501/601 trihalomethanes calibration mix (Supelco, Bellefonte, PA, USA) was used, and the calibration curve included a range of concentrations from 1 µg/L to 1000 µg/L. Results were expressed in mg/L for chlorates and in µg/L for THMs. The compounds quantified were: trichloromethane (chloroform), bromodichloromethane, dibromochloromethane and tribromomethane (bromoform). Presence of cultivable TAB, total coliforms and *E. coli* in process wash water was assessed by membrane filtration (0.45 µm) and surface plating. Serial dilutions were prepared as needed using buffered peptone water (BPW, 2 g/L) (Oxoid, Basingstoke, UK). For assessment of TAB, samples were plated on plate count agar (PCA, Scharlab, Barcelona, Spain) and incubated at 30 °C for 36–48 h. For total coliforms and *E. coli*, Chromocult coliform agar (Merck) and incubation at 37 °C for 24 h were used. Also, an enrichment step was used to assess the presence/absence of *E. coli*. A volume of 50 mL of neutralized sample was mixed with 50 mL of BPW (40 g/L). After incubation at 37 °C for 24 h, enriched samples were streak plated in Chromocult plates. Incubation was performed as described above. For *Enterobacteriaceae*, pour plating in Violet Red Bile Dextrose (VRBD) agar with overlayer was used, incubating at 37 °C for 24 h.

Additionally, samples of the products were periodically taken over time before washing, after the first wash, the second wash, and the final rinse. Presence of DBPs and microbial counts were evaluated in the product samples. DBPs and the microbial groups studied were the same as the ones measured in water samples. *Enterobacteriaceae* were determined in one bell pepper sampling. In the garlic clove samplings, lactic acid bacteria were also examined. Residual water on the surface of each product was removed with a manual centrifuge for 1 min to maintain the same water content as in commercial samples. For the microbiological analyses, a portion (25 g) of each dewatered sample was transferred to sterile stomacher bags and mixed with 100 mL of BPW (2 g/L) containing sodium thiosulphate (0.1 g/L) to neutralize disinfectant residuals. For the detection of TAB, total coliforms, and *E. coli*, pour and surface plating of the homogenized samples were performed using the same media and incubation conditions used for the analysis of wash water. For the detection of *Enterobacteriaceae*, the same media and incubation conditions described for the analysis of wash water were used. For lactic acid bacteria, pour plating with overlayer in MRS+sorbic acid and incubation for 5 days at 22 °C were used. For chlorate analysis, 30 g of centrifuged and non-neutralized fresh produce sample were chopped for 10 seconds using a meat mincer (Moulinex A320; Moulinex, Ecully, France). Ten grams of chopped product were mixed with 10 mL of a solution of formic acid (1%) in methanol. The mixture was sonicated for 2 min, briefly vortexed, and centrifuged (1900 ×g) for 5 min. The supernatant was filtered through 0.22-µm filters (Sartorius Minisart PES; Sartorius, Gottingen, Germany). Then, it was diluted 1:10 using a solution of formic acid (1%) in methanol (Gómez-López et al., 2013). Chlorate content was analyzed by UPLC-MS as described by (Gil et al., 2016); results were expressed in mg/kg fresh weight. For THMs analysis, samples of 1 g of chopped product were transferred to SPME vials and analyzed by GC–MS as explained above (Gómez-López et al., 2013); results were expressed in µg/kg fresh weight.

A large number of lab-scale experiments were performed to determine the operational limits for sodium hypochlorite in different types of fresh produce wash water under controlled conditions. The types of produce wash water studied included that from baby leaves, shredded lettuce, shredded cabbage, and diced onions. These studies were performed using a dynamic system that allows the simulation of the conditions in industrial washing tanks (**Figure 2**; Gómez-López et al., 2013; Gómez-López et al., 2014). In this system, organic matter, microorganisms, and disinfectant entered the water continuously, as in a commercial washing line. Four different FC residual concentrations (≈0, ≈10, ≈20, and ≈30 mg/L) were tested for each wash water. Physicochemical characteristics of wash water were monitored during the experiments, including FC, total chlorine, pH, temperature, ORP, electrical conductivity, COD and UV254. The presence of DBPs such as,THMs and chlorate was also measured.

Data on microbial populations were log-transformed. IBM SPSS statistics 24 was used for statistical analysis. Shapiro Wilk test and Levene's test were used to assessing the normality and the equality of variance, respectively. When normality could be assumed, T-tests were used to compare two treatments. Also assuming normality, One-way ANOVA was done to compare more than two treatments, using Tukey’s HSD or Dunnett’s as post hoc tests depending on the homogeneity of the variances. For data that did not follow a normal distribution, nonparametric tests (Mann–Whitney U and Kruskal–Wallis) were used to search for differences between treatments. Data obtained in all the studies were used to perform correlation analyses between critical water quality parameters. Pearson’s or Spearman’s correlation coefficients were calculated depending on the normality of the data. For the interpretation of results, a correlation between two parameters was considered when the correlation was significant at $\alpha = 0.05$.

Results: Physicochemical parameters

In the different commercial washing lines, in general, the concentration of organic matter increased in the wash water during the sampling period due to the entry of the product. In the case of the washing lines that used PAA, the disinfectant also contributed significantly to the levels of organic matter detected in the water. The dynamics of the organic matter content of wash water were also affected by the type and the cut size of the product washed. Whole products caused a smaller impact on the organic matter levels compared with the cut products. COD is often used as an indicator of the organic content of wash water. In our studies, other parameters including electrical conductivity, UV254, total dissolved solids, and turbidity showed a positive correlation with COD. Interestingly, in most of the tests, independently of the type of product and the disinfectant used, UV254 showed a very strong positive correlation with COD. **Table 3** shows the correlation between COD and UV254 in the different samplings. In two of the samplings performed in fresh-cut lettuce washing lines, no significant COD-UV254 correlation was observed, and this result was due to the lack of change in the COD and UV254 levels. In those samplings, COD and UV254 of wash water remained stable in a narrow range during the whole sampling period, hampering the detection of trends. In the case of peeled garlic cloves disinfected with NaClO, there was also no correlation with COD-UV254. In the garlic wash water treated with chlorine, the COD increased steadily during the sampling period from initial levels of 140 mg/L to final levels of 526 mg/L, while UV254 remained quite stable between 0.15 and 0.22 cm^{-1}. Additional data would be needed to confirm or refute this lack of correlation in garlic cloves wash water. The results suggest that UV254 could be an appropriate parameter for the measurement of the level of organic matter in wash water for most of the process wash waters tested. In most of the samplings, the UV254 analyses were performed using an offline spectrophotometer measurement. However, there is a commercial portable spectrophotometer that could be used by companies to monitor the organic matter content of the wash water. UV254 showed a positive correlation with the populations of TAB and total coliforms, and also with the concentration of DBPs present in the wash water. The data obtained could be used to make evidence-based decisions for water replenishment of the washing systems, with the aim of ensuring a residual concentration of disinfectants and avoiding the accumulation of DBPs.

For the optimal disinfection of wash water, it is critical to maintain the appropriate range of pH when using chlorine to ensure the maximum concentration of hypochlorous acid (HOCl) as the chlorine form with the highest antimicrobial activity. In a lab-scale study using the dynamic system, different pH regulators (citric acid, phosphoric acid, sulfuric acid, and carbonic acid) were evaluated attending to the concentration of HOCl present in chlorinated water at pH range between 3.0–11.0 with sodium hypochlorite as chlorine source. The results showed that the pH range 5.0–6.0 HOCl was the predominant form of chlorine (>90%) for all the pH regulators. Phosphoric acid and sulfuric acid showed a wider optimal pH range (3.0–6.0) to maximize HOCl concentration compared with citric acid and carbonic acid (4.5–6.0 and 5.0–6.0, respectively). Moreover, when citric acid was used as a pH regulator, a decrease in available chlorine was observed at pH <4.5. Additional analyses confirmed the emission of chlorine gas under these conditions. Based on these results, a second set of experiments was carried out to assess the effect of these pH regulators on the DBP generation in the lettuce wash water. Both sodium hypochlorite and calcium hypochlorite were used as chorine sources to maintain FC at 25 mg/L and a target pH of 5.5 using the different pH regulators. All pH regulators did not influence the antimicrobial activity measured as the control of TAB, but some affected some physicochemical characteristics of the wash water, such as chlorine demand and ORP, significantly. When citric acid was used as pH regulator, compared with the other regulators evaluated, higher accumulation of haloacetic acids (HAAs) was observed in lettuce wash water disinfected with sodium hypochlorite, and higher accumulation of THMs when both chlorine sources were used. Chlorate accumulation was not affected by the type of pH regulator, but when sodium hypochlorite was used as chlorine source, the content of chlorate was seven-fold higher than when using calcium hypochlorite. Among the pH regulators, phosphoric acid stood out because of its wide range of pH in which chlorine was present manly in the HOCl form, its inorganic nature that reduces DBPs formation (unlike citric acid), and its less corrosive action to industrial equipment compared with sulfuric acid.

Results: Microbial quality of the wash water and the product

Microbial quality of wash water is affected by different factors, like the concentration of disinfectant, type and cut size of the product washed, water replenishment policy, and the product's throughput. Overall, using the data from all the studies for the correlation analysis, the populations of TAB and total coliforms in the wash water showed negative correlations with the concentration of disinfectants, including FC, PAA, and ClO_2, and also with ORP, whereas there was positive correlation with turbidity (**Table 4**). The results from the study performed in the facilities from one of the industrial collaborators of the project (i.e., bell pepper processing line, PAA as a disinfectant), revealed the consequences of not applying any disinfectant in the fresh produce wash water. In this case, the line comprised two washing steps consisting of roller-brushes combined with showers and waterfall. In the second washing step, in which PAA was applied, the mean number of TAB in the wash water was 0.004% of the amount present in the first wash step in which no disinfectant was used. Furthermore, from the results obtained throughout the project, the impact of the type and cut size of the product in the microbial load of wash water could be assessed. The data obtained in the samplings performed in leafy greens processing lines from one of the industrial collaborators using chlorine suggest that the microbiota present in baby leaves is more resistant to disinfection than the microbiota from fresh-cut lettuce. These results agreed with those for baby leaves processing lines from another industrial collaborator, which showed higher microbial build up in baby leaves wash water than in chopped or shredded lettuce wash water. The microorganisms associated with baby leaves are more exposed to environmental stresses than those of internal leaves from closed-shape leafy greens (e.g., lettuce, cabbage). The tolerance to environmental stresses, such as UV light, can cause tolerance to other stress factors, including the antimicrobial action of disinfectants. Concerning cut size, data from one of the industrial collaborators suggest that the microbial load is higher when shredded lettuce is washed compared with chopped lettuce, probably due to a higher release of organic matter in the water causing a high demand for disinfectant.

In washing lines using chlorine, pH and disinfectant concentration are critical parameters that need to be accurately controlled. In one of the processing plants from one industrial partner, two water disinfection management procedures were compared. One was a poor control of FC and pH, and the other the Automated SmartWash Analytical Platform (ASAP)™ managed by one of the industrial collaborators. This on-line–controlled disinfection system is characterized by the accurate control of FC and pH. **Figure 1** shows the differences in FC and pH observed during the samplings performed in shredded lettuce washing lines. Free chlorine fluctuated between high (maximum=67 mg/L) and almost no FC (minimum=0.9 mg/L) when using the poor control system (**Figure 1A**), while FC was perfectly maintained in the set range of 10–20 mg/L when the ASAP system was used (**Figure 1B** & **1C**). Differences observed between the two types of management of FC and pH had consequences on the efficacy of inactivation of microorganisms in the wash water. The populations of TAB from wash water were significantly lower ($p < 0.05$) when the disinfection was managed by the ASAP system compared with the manual control.

PAA and sodium hypochlorite were evaluated at industrial scale for the disinfection of peeled garlic wash water. Results showed that the microbial load of the wash water was higher when PAA was used. The reasons for these findings were the lower microbial quality of the unprocessed garlic in the PAA trial and the higher amount of peeled garlic washed that made it difficult to compare both disinfectants under those different conditions. One observation from the studies conducted using PAA to wash peppers and peeled garlic was a low bacterial inactivation efficacy in wash water, under the tested conditions.

In general, the washing procedures caused statistically significant reductions in the microbial load of the washed product. The mean reduction obtained varied between the different studies, with a maximum reduction of 2.2±0.5 log in one of the fresh-cut lettuce washing lines, and a minimum reduction of 0.3±0.6 log in the fresh-cut onion washing line. In studies conducted in washing lines of baby leaves, chopped lettuce, and shredded products, a negative correlation was detected between the microbial load of washed product and the FC concentration and ORP of the water. Furthermore, there was a positive correlation between the microbial populations in produce and the pH of the wash water, highlighting the importance of adequate control of pH.

<u>Results: Presence of disinfection by-products</u>

Regarding the occurrence of DBPs in the wash water, chlorate and THMs showed a positive correlation with parameters linked to the organic matter content (COD, UV254, total dissolved solids, electrical conductivity) and total chlorine. The results also revealed significant differences between different chlorine derivatives in the accumulation of chlorate. When chlorine gas alone was used for wash water disinfection, no chlorate was detected in the wash water or the washed product, while when sodium hypochlorite or calcium hypochlorite were used, chlorate was detected both in wash water and the washed products. Water rinse reduced the concentration of DBPs in the washed product significantly, although the reduction was not observed in all the cases due to the variability in the washing schemes between companies, and the need to optimize this rinsing step.

Results: Performance of sensors and test kits under commercial conditions

Additional visits to the companies were devoted exclusively to assess the performance of sensors and test kits for the measurement of disinfectant residuals. Studies focused on the comparison of several sensors for the quantitation of chlorine and PAA.

For chlorine, the performance of various methods for the analysis of FC was tested in washing lines of baby leaves and fresh-cut lettuce in collaboration with an industrial collaborator. Three commercial sensors were compared: (1) Chlorosense from Palintest (Gateshead, UK) based on an electrochemical reaction, (2) Spectroquant from Merck (Darmstadt, Germany) as a DPD-based method, and (3) the test kit Lovibond (Amesbury, UK), also a DPD-based method. The Lovibond method used by the company overestimated the concentrations of FC when compared with ChloroSense and Spectroquant, while no differences were observed between the latter two methods. The test kit used by the company (Lovibond) is highly dependent on operator skills to match the color of the strips with a concentration of FC from a color chart. In contrast, the Chlorosense and the Spectroquant methods are suitable methods to measure the free and total chlorine concentration present in the process wash water accurately.

Although there are several methods for measuring PAA in water, most of them are not appropriate for determining PAA in produce wash water because of the high concentration of PAA used and the possible interference with the organic matter released from the produce. In the case of PAA, the performance of commercial sensors was evaluated in bell pepper, peeled garlic cloves, apple, and lemon wash waters. Three different commercial PAA sensors were compared: (1) Amperometric probe from Dosim (Barcelona, Spain), (2) PAAsense (Palintest) as an electrochemical-based sensor, and (3) Quantofix® test strips from Macherey Nagel (Düren, Germany) as a colorimetric method. It was concluded that the amperometric probe is not an accurate sensor for some types of product wash water, including lemon and peeled garlic wash water. In the case of lemon wash water, organic compounds interfered with the measurements, causing an underestimation of the PAA concentration. In the case of peeled garlic wash water, there was also an underestimation of the PAA concentration measured by the amperometric probe. For apple wash water, the quantification of PAA was carried out with two sensors, the PAASense and a drop count kit (AquaPhoenix Scientific, Hanover, PA, USA) that is based on an iodometric titration method. These two sensors were compared with high-performance liquid chromatography with photodiode array detector (HPLC-DAD) as one of the most accurate and precise analytical techniques. Total organic carbon (TOC) concentration in apple wash water ranged between 0 and 1000 mg/L, and the PAA concentrations tested were 0, 50 and 500 mg/L. Results showed that PAASense provided similar results to those obtained by HPLC for the TOC and PAA concentrations tested, without needing to make dilutions. However, the drop count kit resulted in an overestimation of more than 20% in the PAA concentration. In conclusion, PAASense is a simple, fast and accurate sensor for measuring a wide range of PAA concentrations in wash water with high organic content. In addition to apple washing, in other fresh produce applications with similar conditions, sanitizers, and organic materials, similar outcomes are expected.

Results: Lab-scale studies

Apart from the industrial-scale studies, several lab-scale studies were performed using the dynamic system previously explained. The antimicrobial effect of chlorine was assessed by the inactivation of indigenous total aerobic bacteria (TAB). Based on TAB counts, a lower operational limit for residual FC of 10 mg/L was effective for onion wash water, while 20–25 mg/L was needed for lettuce and cabbage wash waters. However, the levels of TAB in baby leaves wash water were not controlled even when a constant residual FC of 30 mg/L was maintained. The data obtained in these lab-scale tests were in accordance with the results obtained at the industrial scale, and indicate that the microbiota present in baby leaves seems to be more resistant to disinfection than that from cut lettuce. The maximum operational limit for FC based on the concentration of DBPs could not be established in the case of fresh-cut lettuce, onion, and cabbage, as even the lowest FC concentration (10 mg/L) potentially leads to unacceptable levels of chlorate in the product washed (>0.7 mg/kg). In the case of baby leaves, the problem is that the maximum operational limit based on the concentration of chlorate would be 20 mg/L, which is lower than the minimum operational limit based on microbial inactivation that would be above 30 mg/L.

The dynamic system (**Figure 2**) was also used for the validation of critical parameters at lab scale using inoculated foodborne pathogens. Two attenuated *E. coli* O157:H7 strains were used to assess the antimicrobial efficacy of the different disinfectants. Inoculated lettuce wash water and the disinfectant solution were simultaneously introduced in the washing tank. Three treatments were evaluated: sodium hypochlorite (NaClO), peroxyacetic acid (PAA), and chlorine dioxide (ClO_2). For NaClO, pH was maintained in the range 5.6–7.5, and residual concentrations of 1 and 3 mg/L were assessed in wash water with a maximum COD of 500 mg/L. A residual FC concentration of 3 mg/L was effective in inactivating *E. coli* O157:H7, maintaining the population below the limit of detection (1 CFU/mL). A residual PAA concentration of 50 mg/L was not capable of controlling the population of pathogenic microorganisms below the limit of detection (10 CFU/mL) in wash water in tests where the organic matter increased to a maximum COD of 1300 mg/L. For ClO_2, a residual concentration of 1 mg/L was enough for inactivation of the inoculum, as the population of the inoculated pathogen was below to the limit of detection (1 CFU/mL) even with a high organic content (maximum COD = 700 mg/L).

Outcomes and Accomplishments

1. Identification of specific water quality variables suitable as critical parameters and their critical and optimal operational limits for water disinfection under commercial conditions.

<u>Critical parameters for wash water disinfection vary among the different disinfection systems.</u> For chlorine-based disinfectants, including NaClO and $Ca(ClO)_2$, the critical parameters for their microbial efficacy are residual FC, pH, and the content of organic matter. Assuming a proper pH range (5.0–6.0), the lower operational limit for FC concentration depends on the type of product wash water. According to tests performed in controlled conditions at the lab scale, a FC concentration of 10 mg/L is needed for onion wash water (maximum COD 2300 mg/L), 20–25 mg/L for wash water of shredded lettuce and cabbage (maximum COD 900 and 2200 mg/L, respectively), and >30 mg/L for wash water of baby leaves (maximum COD 270 mg/L). The maximum operational limit (based on the concentration of DBPs) for fresh-cut lettuce, onion and cabbage could not be established for NaClO, as even the lowest FC concentration (10 mg/L) can lead to unacceptable levels of chlorate uptake by the product (>0.7 mg/L). In the case of baby leaves, the maximum operational limit of 20 mg/L (based on chlorate concentration) would be lower than the minimum operational limit (>30 mg/L) based on microbial inactivation. However, a tap water rinse can replace the surface water and reduce the residues on the product to some extent. Regarding organic matter content, the lower the concentration the better for lower demand of disinfectants and lower presence of DBPs. Water replenishment operations should be performed to keep the concentration of organic matter as low as possible, taking into account the costs and environmental impact associated with the use of large amounts of cold potable water. A measure of the organic matter level faster than COD is the measurement of UV absorbance at 254 nm. Theoretically, organic matter concentrations above the higher operational limit would be those that led to intolerably high levels of DBPs in the wash water. The maximum concentration of chlorate acceptable in drinking water is 0.7 mg/L (WHO,

2011), and 80 µg/L for THMs (US EPA, 1998). Based on the results obtained, maximum UV254 levels could be used as an indirect measurement of DBPs in the wash water treated with chlorine. For chlorate, all the wash water samples treated with NaClO, independently of the type of product and the UV254 level, presented levels >0.7 mg/L, except those treated with chlorine gas, which does not generate chlorate. For THMs, more research is needed to establish operational limits for UV254 that could be correlated with acceptable/unacceptable levels.

Peroxyacetic acid: Disinfectant concentration is the most critical parameter to ensure the disinfection efficacy of PAA. Based on the efficacy for microbial inactivation in the wash water, higher minimum operational limits than the ones assessed in the project (up to 400 mg/L) should be recommended. Even though the presence of organic matter affects the efficacy of many disinfection treatments, PAA is less affected than other disinfectants (e.g., chlorine, chlorine dioxide). PAA contributes to the increase in organic matter of the wash water. Unlike chlorine, PAA does not generate harmful DBPs when reacting with organic matter, and thus there is no need to establish a higher operational limit on the content of DBPs. Therefore, the content of organic matter in the wash water is not a critical parameter to measure for managing the disinfection of PAA. For PAA, pH is also not a critical parameter as the efficacy is similar in a wide pH range (5.0–9.0). Factors to take into account to establish the higher operational limit for PAA are the possible adverse effects on product quality and the corrosion of the equipment.

2. Validation of critical limits.

The results obtained confirmed that low doses of FC (3 mg/L) and chlorine dioxide (1 mg/L) are capable to efficiently inactivate inoculated pathogenic bacteria in the produce wash water. In contrast, following the same experimental design, in the presence of organic matter, PAA (50 mg/L) was not as effective as FC (3 mg/L) and chlorine dioxide (1 mg/L) in the inactivation of pathogenic microorganisms. It should be taken into account that these tests were performed using inoculated pathogenic microorganisms that could behave differently than those naturally present in contaminated produce. Furthermore, these tests were performed with vegetative forms of pathogenic bacteria, which are more susceptible to disinfection than other microbial agents (bacterial spores, protozoa, viruses).

3. Performance of commercial sensors and test kits.

For measurement of FC, Chlorosense as an electrochemical method by Palintest, and the test strip Spectroquant as a DPD-based method by Merck (Darmstadt, Germany) were the most reliable methods in leafy greens wash water (fresh-cut lettuce and baby leaves). Also, Chlorosense requires less sample dilution and it measures total chlorine, which can be relevant to estimate risk of DBPs.

For PAA, the commercial sensor PAASense (Palintest) was suitable for measurement of the concentration of PAA in different types of produce wash water. It demonstrated high reliability in the tests performed in washing lines of bell pepper, peeled garlic, apples, and lemons.

4. Verification of critical and operational limits.

Based on lab-scale results, a lower operational limit of 20 mg/L for residual FC was suggested in the case of cut lettuce wash water. This concentration was efficient in reducing the population of TAB below 2 log cfu/100 mL. The suitability of this limit was verified at industrial scale in the facilities of one industrial collaborator. The recommended residual FC of 20 mg/L together with a proper pH (5.0–6.0) maintained the microbial quality of the wash water under the tested conditions of high inflow of organic matter and microorganisms from shredded lettuce. The populations of TAB were maintained below 3 log cfu/100 mL in the commercial washing line when fresh-cut lettuce was washed.

The microbiota of baby leaves showed high resistance to chlorine, and FC levels above 30 mg/L would be needed in the wash water. Accordingly, under industrial conditions even with residual FC levels above 40 mg/L (pH 7–8.5), populations of TAB >4 log cfu/100 mL were detected in the wash water. When residual levels of FC were maintained above 50 mg/L (pH 6.5–8.0) in the wash water of baby leaves, the mean population of TAB was around 2 log cfu/100 mL.

According to the lab-scale tests, a residual FC of 10 mg/L is enough to keep the microbiota of onion wash water below 2 log cfu/100 mL, with maximum COD values of 2300 mg/L. However, in contrast with the lab-scale results, high populations of TAB (>3.9 log cfu/100 mL) were detected in industrial wash water of diced onion, with high levels of FC (100–150 mg/L) and maximum COD values of 2300 mg/L. This discrepancy could be explained by the higher pH level maintained in the industrial wash water (range 7.5–9.3).

5. Elaboration of evidence-based standards.

Operational standards of produce wash disinfection systems have been compiled in **Annex 1** (included at the end of this report). Operational standards are those science-based standards recognized as critical to be monitored for continuous improvement and control of the disinfection process. In the fresh produce industry, they relate to the precise criteria designed for the correct use of disinfection practices. These operational standards are intended to be a summary of best practices rather than general practice. The standards included in **Annex 1** are created by bringing together the experience and expertise of scientists and processors as well as disinfectant companies.

Annex 1 is organized into four parts. Part 1 discusses a series of key points that can be followed for the selection of the disinfection with chlorine. Part 2 deals with applying the operational standards for chlorine dioxide as one of the disinfection systems more demanded. Part 3 focuses on measuring and evaluating the operational standards for PAA, and part 4 is concerned with actions to improve the performance of the disinfection system regarding DBPs.

Summary of Findings and Recommendations

The critical parameters that affect the performance of different wash systems (defined by factors like the disinfectant used and the product washed) were determined. The operational limits for the different parameters in different wash systems were established. Suitable methods for the measurement of the selected parameters were assessed and selected. The efficacy of the established operational limits for the inactivation of pathogenic microorganisms was verified. Finally, evidence-based standards for different wash systems were proposed.

In the case of chlorine, residual FC and total chlorine, pH, and organic matter would be the most critical parameters. The organic matter, on-line monitoring of UV254, which correlates with the COD, could be used for many types of produce wash water. In the case of PAA, the level of disinfectant is the only critical factor to be controlled, as organic matter and pH are not related to the disinfection efficacy. Water replenishment is necessary to reduce the volume of sanitizer added and dilute the organic matter accumulated in the wash water, which directly affects the formation and accumulation of DPBs.

The lower operational limit for FC varies between different types of produce wash water. It ranges from 10 mg/L (for onion wash water), to >30 mg/L for wash water of baby leaves. The lower operational limit for PAA would be higher than 200 mg/L for peeled garlic, and higher than 400 mg/L for bell peppers.

The commercial electrochemical sensor PAASense (Palintest, Gateshead, UK) is suitable for measuring PAA in a wide range of concentrations in produce wash water with high organic content. The electrochemical method Chlorosense (Palintest), and a DPD-based method (Spectroquant, Merck, Darmstadt, Germany) are appropriate sensors for FC and total chlorine.

APPENDICES

Publications

López-Gálvez, F., Tudela, J.A., Allende, A., Gil, M.I. 2018. Microbial and chemical characterization of commercial washing lines of fresh produce highlights the need for process water control. Innovative Food Science and Emerging Technologies, *In Press*, DOI 10.1016/j.ifset.2018.05.002.

Gil, M.I., López-Gálvez, F., Andújar, S., Moreno, M., Allende, A. 2019. Disinfection by-products generated by sodium hypochlorite and electrochemical disinfection in different process wash water and fresh-cut products and their reduction by activated carbon. *In Press*. Food Control.

Garrido, Y., Marín, A., Tudela, J.A., Allende, A., Gil, M.I. 2019. Chlorate uptake during washing is influenced by product type and cut size, as well as washing time and wash water content. *Submitted to Postharvest Biology and Technology.*

Tudela, J.A., López-Gálvez, F., Allende, A., Gil, M.I. 2019. Concepts on the disinfection of fresh-cut produce wash water using liquid chlorine and chlorine gas illustrated with data obtained at industrial scale. *Submitted to Food Control.*

Tudela, J.A., López-Gálvez, F., Allende, A., Hernández, N., Andújar, S., Marín, A., Garrido, Y. Gil, M.I. 2019. Assessment of operational limits for fresh-cut produce wash water disinfection with sodium hypochlorite. *Submitted to Food Control.*

Tudela, J.A., Garrido, Y., Marín, A., Hernández, N., Andújar, S., Pérez Sosa, B., Haway Caballero, A., Allende, A., Gil, M.I. Selection of pH regulators for chlorinated wash water of fresh produce based on disinfection by-products and chlorine gas production. *In preparation*.

Albolafio, S., Tudela, J.A., Hernández, N., Allende, A., Gil M.I. Selection of sensors for monitoring and control of peroxyacetic acid in produce wash water. *In preparation*.

López-Gálvez, F., Truchado, P., Tudela, J.A., Gil, M.I., Allende, A. 2019. Use of peroxyacetic acid in a commercial bell pepper washing line: Microbial quality of water and produce, assessment of disinfectant residuals. *In preparation.*

Presentations

Allende, A. Establishment of operating standards for fresh produce wash systems. Organized by Kronen at the 40th Aniversary. Kehl-Goldscheuer (Germany) 28 September 2018.

Allende, A. HUPlantControl COST Action 16110 Conference. Risk posed by pathogens in food of non-animal origin: What is going on lately at the CEBAS-CSIC? Berlin, Ger., March 2018.

Allende, A. National Association of Tropical Fruits. V Annual meeting. May 2018. Motril, Granada: Optimización en los recursos de la producción. Title: Aguas regeneradas para uso agrícola: Beneficios y perjuicios.

Allende, A. The International Conference on Food Innovation. Foodinnova 2017: “Emerging and common safety issues associated to fresh produce”. Cesena, Italy. 31 Jan – 3 Feb 2017.

Allende, A. Workshop Internacional Procesado de Vegetales Frescos – Juan Neustadtel S.A.S. XXI Agroexpo corferias 2017. Gestión del agua en la producción y procesado de productos vegetales: oblemas y posibles soluciones. Bogotá, Colombia, 13 July 2017.

Allende, A. Workshop CLODOS Technology, Desinfección sin límites : “Tratamiento de agua de riego con Agridis: dióxido de cloro estabilizado”. Almería, Spain, 12 May 2017.

Allende, A. International Association for Food Protection Conference 2017. Tampa, Florida. 11-12 July 2017. Titled: The microbial ecology of fresh produce: Can behavior of resident bacteria affect transient colonizers?. Symposium “Fresh Produce and Pathogen-Pairs in the US and Europe.”

Allende, A. Jornada de Seminarios IATA, Valencia. Title: Gestión del agua en la producción y procesado de productos vegetales: Problemas y Posibles Soluciones. Valencia, Spain. 26 May 2017.

Allende, A. XIII JORNADAS TÉCNICAS DE SANEAMIENTO Y DEPURACIÓN. ESAMUR. Title: Buenas Prácticas Agrícolas y la relevancia de la evaluación cuantitativa de riesgos en la predicción de peligros biológicos en la toma de decisiones. Murcia, Spain, 16–17 Nov 2017.

Allende, A. Jornadas de higiene de la producción primaria agrícola y su control oficial MAPAMA. Title: Puntos críticos a controlar relacionados con la higiene en explotaciones. Medidas de mitigación y actuación ante presencia de microorganismos patógenos. Madrid, Spain. 7–8 Nov 2017.

Gil, M.I. Postharvest research and industry implications. Keynote International "VI Postharvest Unlimited", Fruit Attraction, Madrid, October, 2017.

Gil, M.I. Aguas de lavado en la industria de vegetales: Riesgos microbiológicos y químicos. CAF. Banco de Desarrollo de América Latina. Misión empresarial y visita al CEBAS-CSIC. November, 2017.

Gil, M.I. Chemicals in the salad bag: who knows what they put in there. Workshop: Fresh-cut products: Scientific approach vs fake news. MacFrut, Rimini (Italia), May, 2018.

Gil, M.I. Panorama actual y tendencias en el mercado de IV y V Gama. XII Simposio Nacional y X Ibérico de Maduración y Postcosecha, SECH, Badajoz, Spain, June, 2018.

Gil, M.I. Establisment of operating standards for fresh produce wash systems. Kronen Anniversary, Kehl-Goldscheuer (Germany) September, 2018.

Gil, M.I. 9th European Short-Course on Quality and Safety of Fresh-cut Produce. "Water management in fresh-cut operations". Oporto, Portugal, October, 2018.

Gil, M.I. Training Network Course on Biotechnology, Physiology and Plant Patology. "Disinfection methods for process wash water". November, 2018.

Budget Summary

Total funds awarded were $248,521.40, and all funds are expected to be used.

	Q1	Q2	Q3	Q4	TOTAL YEAR 1
Supplies	$3,623.76	$8,251.94	$75.20	$1,566.81	**$13,517.72**
Personnel	$2,670.01	$17,269.30	$16,149.41	$20,960.34	**$57,049.06**
Others	$32.02	$0.00	$792.35	$0.00	**$824.37**
Travel	$0.00	$5,270.12	$0.00	$45.21	**$5,315.34**
Indirect costs	$160.20	$1,036.16	$968.96	$1,354.34	**$3,519.67**
TOTAL	$6,486.00	$31,827.53	$17,985.93	$23,926.71	**$80,226.15**
	Q1	**Q2**	**Q3**	**Q4**	**TOTAL YEAR 2**
Supplies	$6,666.16	$5,804.90	$17,627.37	$37,977.69	**$68,076.12**
Personnel	$16,829.38	$20,571.93	$16,299.52	$24,509.06	**$78,209.89**
Others	$1,059.81	$44.49	$1,681.38	$0.00	**$2.785.68**
Travel	$831.35	$7,595.88	$923.08	$485.33	**$9,835.64**
Indirect costs	$1,009.76	$1,234.32	$977.98	$1,470.54	**$4,692.60**
TOTAL	$26,396.46	$35,251.52	$37,509.33	$64,442.63	**$163,599.94**

Tables 1–4 and Figures 1–2

Table 1. Summarized information of the samplings performed.

Plant material	Product size	Disinfectant	Number of samplings
Bell pepper	Whole	PAA	4
Baby leaves mixes	Whole	$NaClO+Ca(ClO)_2$	1
Fresh-cut lettuce[a]	Cut	$NaClO+Ca(ClO)_2$	1
Shredded products[b]	Cut	$NaClO+Ca(ClO)_2$	1
Onion	Cut	NaClO	1
Fresh-cut lettuce[c]	Cut	NaClO	1
Leafy greens[d]	Whole/Cut	NaClO	3
Tomato	Whole	ClO_2	2
Fresh-cut lettuce[c]	Cut	Cl_2	2
Fresh-cut lettuce[c]	Cut	$NaClO+Cl_2$	3
Peeled garlic cloves	Whole	PAA	1
Peeled garlic cloves	Whole	NaClO	1
Total Samplings			21

[a]Romaine and Frisee; [b]Carrot, red cabbage; [c]Iceberg; [d]Baby leaves mixes+fresh-cut lettuce (Iceberg and Romaine).

Table 2. Characteristics of the different washing lines evaluated.

Product	No. of washing steps	Steps with disinfectant	Application	Tap water rinse
Bell pepper	2	1	Roller Brushes+Showers+Waterfall	No
Baby leaves mixes	2	2	Immersion open tank+bubble	Yes
Fresh-cut lettuce[a]	2	2	Immersion open tank+bubble	Yes
Shredded products[b]	2	2	Immersion closed system	Yes
Onion	2	2	Immersion open tank	Yes
Fresh-cut lettuce[c]	2	2	Immersion open tank+bubble	Yes
Leafy greens[d]	2	2	Immersion open tank+bubble	Yes
Tomato	1	1	Roller Brushes+Showers	No
Fresh-cut lettuce[c]	1	1	Immersion open tank+bubble	Yes
Peeled garlic cloves	1	1	Immersion open tank	Yes

[a]Romaine and Frisée; [b]Red cabbage, carrot; [c]Iceberg; [d]baby leaves mixes and fresh-cut lettuce.

Table 3. Correlation (Pearson's correlation coefficient, PCC) between chemical oxygen demand (COD) and absorbance at 254 nm (UV254) for different combinations of disinfectant-product.

Plant material	Product size	Disinfectant	PCC COD-UV254
Bell pepper	Whole	None	0.90
Bell pepper	Whole	PAA	0.88
Various[a]	Whole/Cut	NaClO+$Ca(ClO)_2$	0.96
Onion	Cut	NaClO	0.90
Lettuce	Cut	NaClO	0.99
Lettuce	Cut	NaClO	0.94
Leafy greens	Whole/Cut	NaClO	0.96
Leafy greens	Whole/Cut	NaClO	0.98
Leafy greens	Whole/Cut	NaClO [b]	0.98
Leafy greens	Whole/Cut	NaClO [b]	0.94
Leafy greens	Whole/Cut	NaClO [b]	0.99
Leafy greens	Whole/Cut	NaClO [b]	0.99
Tomato	Whole	None	0.94
Tomato	Whole	ClO_2	0.99
Lettuce	Cut	Cl_2	0.93
Lettuce	Cut	NaClO+Cl_2[b]	0.89
Lettuce	Cut	NaClO+Cl_2[b]	0.97
Lettuce	Cut	Cl_2	NS
Lettuce	Cut	NaClO+Cl_2[b]	NS
Peeled garlic cloves	Whole	PAA	0.97
Peeled garlic cloves	Whole	NaClO	NS

[a] Baby leaves, fresh-cut lettuce, shredded carrot, and red cabbage; [b]Operated by Smartwash Solutions™; NS: not significant.

Table 4. Main correlations detected between microbial and physicochemical parameters using data from all the studies. ORP: Oxidation-reduction potential. PAA: peroxyacetic acid. N: the number of observations used for the analysis. α: Significance of the relationship between the two variables.

Microbial parameter	Physicochemical parameter	Spearman's rho	N	α
TAB	Chlorine dioxide	-0.895	9	$1.1 \cdot 10^{-3}$
	ORP	-0.709	178	$1.5 \cdot 10^{-28}$
	PAA	-0.699	62	$2.7 \cdot 10^{-10}$
	FC	-0.549	112	$3.6 \cdot 10^{-10}$
	Turbidity	0.604	128	$4.5 \cdot 10^{-14}$
Total coliforms	Chlorine dioxide	-0.876	9	$1.9 \cdot 10^{-3}$
	PAA	-0.797	62	$9.8 \cdot 10^{-15}$
	FC	-0.696	112	$1.5 \cdot 10^{-17}$
	ORP	-0.624	178	$1.4 \cdot 10^{-20}$
	Turbidity	0.624	128	$3.5 \cdot 10^{-15}$

Figure 1. Free chlorine (mg/L), hypochlorous acid (HClO) (mg/L), and pH of shredded lettuce wash water in samplings performed in shredded lettuce washing lines from an industry partner. A, B & C: Water disinfection manually managed by the industry operator. D, E & F: water disinfection managed by the ASAP^{TM} system.

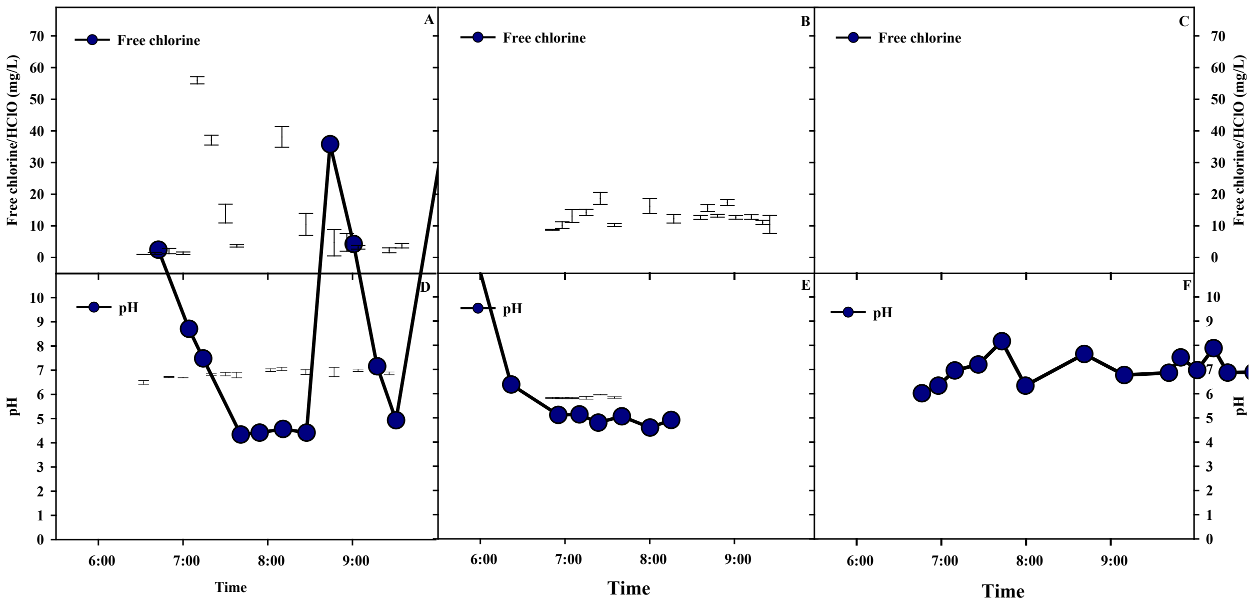
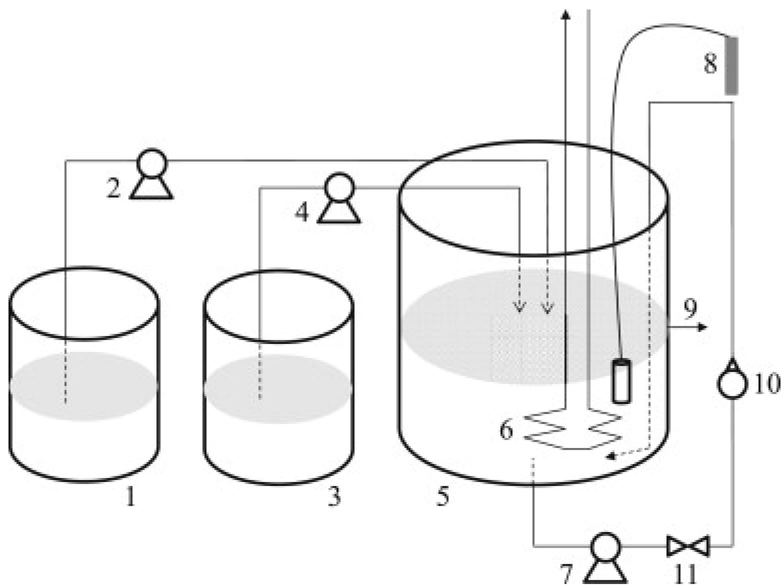


Figure 2. Lab-scale dynamic system. 1: reservoir polypropylene tank for inoculated process water; 2 & 4: peristaltic pumps for dosing; 3: reservoir polypropylene tank for the disinfectant agents; 5: stainless steel tank (30 L); 6: cooling system; 7: centrifuge pump for water recirculation; 8: control board with display sensor for pH, redox potential and temperature; 9: overflow valve; 10: rotameter; 11: flow control valve.



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ANNEX 1

Operational Standards of Produce Wash Systems

This guide is designed to help producers to adopt knowledge developing and implementing specific metrics and methods of analyses for process water treatment.

Introduction

What are operational standards?

Operational standards are science-based criteria critical enough to be implemented for continuous improvement and control of the process water treatment. In the fresh produce industry, they relate to the precise criteria designed for the correct use of processing aids, antimicrobial water agents or sanitizers.

Operational standards help to increase the reliability of process water practices and are intended to be a summary of best practices rather than a description of regular practice. These standards are created by bringing together the experience and expertise of scientists, produce processors, and disinfectant manufacturers.

Selection of a process wash water treatment

Postharvest washing is one of the most critical operations that may compromise food safety. Fresh fruits and vegetables are often washed to remove dirt from the field before distribution. Most processors recirculate process wash water to save water and energy. By doing that, organic matter from the dirt and soil present on the vegetable surface, and the organic matter released from the damaged areas of the produce accumulates in the water.

The water of inadequate microbiological quality has the potential to be a direct source of contamination and a vehicle for spreading pathogens. Therefore, wash water is treated with chemical antimicrobials to prevent cross-contamination. The efficacy of wash water treatment must be monitored and controlled throughout the establishment of operational limits. For the antimicrobial agent, if the concentration is not maintained correctly, it usually declines rapidly in the washing operation as a result of its reaction with soluble organic materials present in the wash water. The concentration of residual antimicrobial is considered the most critical control factor in produce washing systems. However, other factors can affect the performance of the process water treatment. The organic matter content can affect the concentration of the processing aid, its antimicrobial action, and its precise measurement, making it difficult to control. Therefore, the efficacy of antimicrobials for the wash water treatment is defined by a combination of factors.

Traditional testing methods for determining the levels of antimicrobial agents in the wash water involve using portable colorimetric methods. However, these methods are known to have several problems when used in the fresh produce industry, which include i) a lack of specificity (e.g., not being able to easily determine free chlorine as opposed to combined chlorine, specifically at hyperchlorination levels), ii) the complexity of the test, and iii) the use of glassware and chemical reagents that are not appropriate in the food production environment.

Key points about process wash water

☐Wash water is widely used in handling and processing facilities for washing fresh fruits and vegetables. To reduce water and energy costs, in most of the cases, water is recirculated or re-used. Dirt, organic matter, chemicals, and disease-causing pathogens coming from the vegetable surface, are accumulated in the process water over time.

☐Biological and chemical contaminants released from the product to the process wash water may cross-contaminate non-contaminated product entering the washing tank. The microbiological quality of process water must be maintained using antimicrobial treatments to avoid cross-contamination with biological agents (e.g., foodborne bacteria and viruses).

☐Each washing system is different because of the process design, type of produce and cut size as well as water treatment. A case-by-case study is needed to tailor the specific wash water quality parameters and operational limits for each particular product and sanitizer.

☐Optimizing the concentration of disinfectant during produce washing to ensure water quality and prevent cross-contamination represents a challenge for the industry.

☐ Water replenishment is an important issue to control in the washing processes. The physicochemical quality of process water determines the replenishment. A fast measurement of the organic matter content is needed to identify when water replenishment is needed to reduce the disinfectant demand and the accumulation of disinfection by-products (DBPs).

☐The establishment of operational limits for the concentration of the antimicrobial agent is necessary: the lower operational limit must be based on the inactivation of microorganisms and the higher operational limit should be established to minimize the presence of DBPs. Depending on the product, these limits can vary.

☐To monitor and control the established operating standards, selection of appropriate sensors is necessary. Many commercial sensors are useless because of the interference with the organic matter present in the process water.

☐Available commercial on-line systems for monitoring and control critical parameters of process water are strongly recommended. Most focus on free chlorine, pH, and ultraviolet absorbance at 254 nm (UV254).

This guide is organized into four parts. Parts 1, 2, and 3 discuss a series of key points that processors can follow for the optimization of process water treatment with chlorine, chlorine dioxide, and peroxyacetic acid, respectively. Finally, part 4 is concerned with actions processors can take to improve the performance of the selected water treatment regarding disinfection by-products.

Part 1: Operational Standards for Chlorine

1.1 Main characteristics

Chlorine, as sodium or calcium hypochlorite and to a lesser extent as chlorine gas, is the main processing aid used in horticultural sanitation programs.

Electrolyzed water is a promising alternative to sodium hypochlorite, although under high organic matter demand a high chlorine residual is challenging to generate.

A proper pH range is critical to maximize the concentration of hypochlorous acid to achieve the highest antimicrobial activity.

When using chlorine, a pH regulator is needed because hypochlorous acid, the active form of chlorine, predominates at pH 5.0–6.0. Among the pH regulators tested:

- Citric acid causes chlorine gas emission at pH <4.5 and contributes to the accumulation of DBPs in chlorinated wash water due to its organic acid nature.
- Phosphoric acid is the best as it maximizes the presence of hypochlorous acid in a wider pH range without chlorine gas emission or DBP contribution.
- Sulfuric acid is corrosive to the equipment.
- Carbonic acid is difficult to control and the lower pH that can be achieved is 5.0.

The presence of organic compounds can lead to a rapid depletion of hypochlorous acid, diminishing the antimicrobial capacity of chlorine.

Chlorine derivatives contribute to the formation and accumulation of disinfection by-products (DBPs) in the wash water. Organic DBPs are generated by the oxidation of organic compounds, and the main ones are trihalomethanes (THMs) and haloacetic acids (HAAs). Inorganic DBPs are formed due to the chemical instability of chlorine, and include chlorate and perchlorate.

Chlorate is the main DBP of sodium hypochlorite. When using hypochlorite and electrolyzed water, chlorate concentrations in process water are usually smaller, while chlorine gas does not generate chlorate.

1.2 Critical parameters

Critical parameters include chlorine concentration, the pH of the process water, and the organic matter content of the process water.

That the chlorine efficacy is improved by monitoring and controlling free chlorine and pH is well known. Measuring process water quality based on the organic matter content as a critical parameter is recommended for controlling the microbial quality of the process water and the potentially accumulated DBPs. The COD measure is linked to chlorine demand and DBP formation but as it takes time and tedious protocol, other parameters have been identified as useful to monitor changes in the organic matter content. Within this regard, UV254 has been suggested as a good indicator mostly because of its good correlation with COD.

The relation between process water properties and organic load/chlorine demand may be different for each type of produce wash water.

1.3 Specific metrics

Phosphoric acid is the pH stabilizer recommended to maintain pH in the range 5.0–6.0 to maximize the concentration of hypochlorous acid. Citric acid is no longer recommended.

Recommendations of residual free chlorine are difficult because depending on the type of product, the release of organic matter is different, and this directly affects the chlorine demand.

It is recommended to maintain constant free chlorine of 3 mg/L as a lower operational limit to prevent the risk of cross-contamination for a low demand process water.

Constant free chlorine between 10–20 mg/L can be recommended for process water of diced onions, chopped and shredded lettuce, and shredded carrots and cabbage. Free chlorine levels higher than 40 mg/L are needed in some cases, such as baby leaves.

To limit accumulation of chlorate in process water to prevent the issues of uptake by the product two alternatives can be followed: 1) the selection of chlorine source and the use of fresh and diluted hypochlorite solutions; and 2) to use a prewash step by means of a shower for fresh-cut products to reduce the organic matter in the washing tank.

1.4 Methods of analysis

- **On-Line Measurement Controllers** are very useful to monitor the level of free chlorine in the wash water.

Oxidation-Reduction Potential (ORP) Sensors. These on-line controllers measure the redox potential. Sometimes, it is combined with pH measure as well. Methods based on ORP are not precise enough as an operational standard to ensure that the residual free chlorine level is maintained. The direct measurement of free chlorine combined with an operational limit at 650 mV ORP can be adequate in meeting microbial reduction and prevention of cross-contamination. There are some exceptions such as process water of baby leaves processing lines where we were able to monitor ORP of 770 mV and 30 mg/L of free chlorine but still show microbial concentration of 5 log of total aerobic bacteria. One disadvantage of the on-line ORP sensor is that it can get saturated and the response over time be diminished to correct the dosing.

DPD Colorimetric Sensors. DPD colorimetric detection for chlorine measurement is based on N,N-diethyl-p-phenylenediamine (DPD), which reacts with the active free chlorine to form a colored product that is read photometrically to determine the amount of chlorine present (**Figure 1**).

Figure 1. On-line DPD analyzer.

The standard on-line DPD analyzer uses small valves and peristaltic pumps to deliver specific amounts of process water, DPD reagent and buffer solution to the measuring cell. The operating range for DPD reagent measurement is 0–5 mg/L free (or total) chlorine. The sensor can be calibrated on-site using either a standard solution or by adjusting the reading to match an external test.

Amperometric Sensors. An amperometric sensor design consists of two electrodes (anode and cathode), a membrane and fill solution (**Figure 2**). These sensors measure a change in current caused by the chemical reduction at the cathode. A membrane and electrolyte help to control the reaction. Constant flow rate and pressure are controlled to ensure an accurate measurement. The operating principal is the diffusion of hypochlorous acid through the membrane and its electrochemical reduction, which is proportional to the chlorine concentration. The compensation for the pH of the sample is needed. High turbidity and organic matters in the process water cause interferences. Calibration of the sensors is required and validation of the measurements by comparison with another hand-held test is recommended.

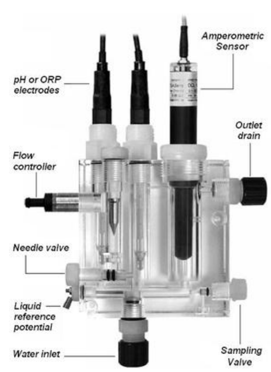

Figure 2. Amperometric sensor.

- **Test methods**. There are other practical testing methods for chlorine measurement to calibrate and validate on-line controllers. These methods should be simple, accurate, rapid, and inexpensive.

ChloroSense (Palintest Water Analysis Technologies, Gateshead, UK) is a portable instrument for accurate measurement of free and total chlorine, with US EPA approval (**Figure 3**). It is a sensor technology for measuring free chlorine (0–10 mg/L) and total chlorine (0–100 mg/L). There is a new version of this instrument (ChloroSense HR) that allows the measurement of higher concentrations of free chlorine (up to 25 mg/L) and total chlorine (up to 500 mg/L). This technique is based on chronoamperometry with repeatable and reliable measurements, which is suitable for a wide range of produce water types and free chlorine concentrations. Chronoamperometry involves applying a fixed voltage to an electrode and recording the resulting current over time. The magnitude of the current is proportional to the level of chlorine in the test sample. The sensor technique is not affected by solids or sample color.

Figure 3. ChloroSense test device.

Spot checks on on-line controller efficacy are usually carried out using a portable method such as a colorimeter. However, the ChloroSense is highly recommended as a secondary test method to calibrate on-line controllers as well as to detect any problems with the on-line controller.

Spectroquant® Chlorine test (Merck, Darmstadt, Germany) is a DPD-based method for measuring free and total chlorine in a rapid and simple analysis (**Figure 4**). In weakly acidic solution, free chlorine reacts with dipropyl-p-phenylenediamine (DPD) to form a red-violet dye that is determined photometrically. The method is analogous to EPA 330.5, APHA 4500-Cl2 G, and DIN EN ISO 7393-2. This method is US EPA approved for drinking water.

Figure 4. Spectroquant® Chlorine test

Palintest chlorine test uses the DPD method in which the reagents are provided in tablet form for maximum convenience and simplicity of use. Free chlorine reacts with diethyl-p-phenylenediamine (DPD) in a buffered solution to produce a pink coloration. The intensity of the color is proportional to the free chlorine concentration. Subsequent addition of excess potassium iodide induces a further reaction with any combined chlorine present. The color intensity is now proportional to the total chlorine concentration; the increase in intensity represents the combined chlorine concentration. In this way, it is possible to differentiate between free and combined chlorine present in the sample. The color intensities are measured using a Palintest Photometer.

Figure 5. Palintest chlorine test that uses the DPD method.

1.5 Commercial products

The company SmartWash Solutions has developed an Automated SmartWash Analytical Platform (ASAP)™ with a Pinpoint Calibration System™ to verify the consistent implementation of chlorine disinfection (**Figure 6**). The system uses high-tech data processors (Process ProData Live, PPDL™) to monitor the chemistry of the process water in real time. The company has patented a processing aid recognized as safe that comprises phosphoric acid and propylene glycol to optimize chlorine-based disinfection of water, to mitigate bacterial cross-contamination and assure optimal food safety.

Figure 6. SmartWash System (http://es.smartwashsolutions.com/#safety-anchor)

Part 2: Operational Standards for Chlorine Dioxide

2.1 Main characteristics

Chlorine dioxide (ClO_2) is an alternative antimicrobial agent to chlorine for process water treatment. It can be generated on-site or used as stabilized aqueous solutions of ClO_2.

Chlorine dioxide overcomes some of the disadvantages of using chlorine. It is a strong oxidizer but it does not generated DBPs upon reaction with organic matter. Its efficacy has a small dependence on pH in the range 5–9. Careful control of the pH of the process water is not needed considering the pH of produce wash water. Furthermore, chlorination demand is higher than ClO_2 demand, resulting in a lower ClO_2 dose to maintain a residual level.

As previously mentioned, one important characteristic is that ClO_2 does not generate THMs. However, one drawback of the use of chlorine dioxide is the accumulation of undesired inorganic by-products (chlorite and chlorate ions). During produce washing, organic matter is continuously introduced in the process water, requiring continuous antimicrobial dosing that causes a constant buildup of chlorite and chlorate.

2.2 Critical parameters

The antimicrobial action of ClO_2, compared with that of chlorine, is less affected by the presence of organic matter. However, organic matter concentration is a critical parameter to measure when using ClO_2, because with a higher disinfectant demand, a higher accumulation of DBPs occurs.

Furthermore, the residual concentration is another critical parameter that needs to be controlled to guarantee the efficacy of the treatment with ClO_2. The level of aeration of the water (jacuzzi systems, sprays, splashing) is critical when using ClO_2, as an excess of aeration leads to the release of ClO_2 to the environment. Release of ClO_2 hinders the maintenance of the concentration in the water and is hazardous for the workers.

2.3 Specific metrics

It is recommended to maintain a constant concentration of ClO_2 with a minimum operating limit of 1 mg/L to prevent the risk of cross-contamination.

There is no need to regulate pH as long as it is in the range 5.0–9.0.

If high concentration of organic matter is present, it can be used as it is not as reactive with organic matter as chlorine. However, high organic matter content will lead to high ClO_2 demand and the accumulation of inorganic DBPs.

2.4 Methods of analysis

- **On-Line Measurement Controllers**. Similar to chlorine, ORP sensors can measure ClO_2 concentrations less than 3 mg/L although it does not specifically measure residual concentration, it responds to all oxidation reduction reactions present and is indicative of free sanitizer available. The amperometric ClO_2 Sensor is an on-line sensor that uses a membrane that allows the selective diffusion of ClO_2 molecules to the amperometric sensor (**Figure 7**).

Figure 7. Hach Chlorine Dioxide Amperometric Sensor (https://www.hach.com/9187-sc-chlorine-dioxide-amperometric-sensor/product?id=7640294023).

- **Test methods: ChlordioXense** (Palintest) is a portable instrument for the accurate measurement of chlorine dioxide in water, based on an electrochemical technique known as chronoamperometry (**Figure 8**). A fixed voltage is applied to a working electrode, and the resulting current-time dependence is recorded by the device through a disposable sensor. The ChlordioXense precisely determines the exact ClO_2 concentration. The instrument display gives a direct reading of the test result in mg/L and it can be used to measure ClO_2 concentrations in the range 0.02–50 mg/L.

Figure 8. ChlordioXense device (Palintest).

2.5 Commercial products

The company Servicios Técnicos de Canarias (STC, Las Palmas de Gran Canaria, Spain) has developed CLODOS Technology® that includes trade marks like AGRI DIS® that can be used for process water treatment. It is a stable aqueous solution of highly concentrated ClO_2 (7500 mg/L). It is not corrosive for the industrial equipment and prevents the formation of biofilms.

Part 3: Operational Standards for Peroxyacetic Acid

3.1 Main characteristics

Peroxyacetic acid or peracetic acid (PAA) is a water-soluble oxidant with great potential as an antimicrobial agent for produce wash water treatment.

PAA is supplied as an equilibrium mixture with varying levels of PAA, acetic acid and hydrogen peroxide, depending on the commercial product.

Regardless of the levels of the other components in the mixture, PAA is the antimicrobial substance considered for wash water treatment.

Being less reactive than hypochlorite, PAA may survive longer time in contact with organic. However, the effectiveness of PAA is highly dependent on the type of produce being washed.

Easy to implement without the need for significant capital investment.

Absence of disinfection by-product formation.

Its efficacy is quite stable at pH lower than 9 and can function over a wide range of temperatures.

Drawbacks: Instability at concentrations higher than 15% (w/v), higher cost compared with chlorine-based disinfectants and slower microbial inactivation kinetics compared with chlorine.

Safety: no danger when diluted in water to their effective concentration. In concentrated solution, it must be treated with caution as a strong and highly corrosive oxidant.

3.2 Critical parameters

PAA concentration is the main parameter to measure for the control of process water treatment. ORP cannot be used for proper control of the dosage of PAA solutions into the process water.

One of the disadvantages associated with PAA is the increase in the organic content in the treated process water due to acetic acid already present in the PAA mixture as well as the acetic acid generated after the PAA decomposition. These characteristics make difficult to control the accumulation of organic matter in process wash water difficult by using COD and/or Abs254 measurements.

Monitoring of pH is not necessary.

3.3 Specific metrics

PAA is EPA approved for use in fruit and vegetable applications (such as process water) and surface applications (such as sanitation of food contact surfaces). FDA does not permit PAA to exceed 80 ppm in process water (21 CFR 173.315). However, in Spain, authorized commercial PAA products, such as Citrocide Plus (Citrosol, Potríes, Spain), are allowed for treatment by shower or immersion in tomato and bell peppers at the recommended dose of 300 mg/L PAA. Fruits must be subjected to a final rinse with potable water before commercialization.

Maintain constant PAA concentration by monitoring.

Low impact of pH and temperature on the efficacy.

If high concentration of organic matter is present, it can be used as it is not as reactive with organics as chlorine.

3.4 Methods of analysis

- **On-Line Measurement Controllers**. Precise, real-time amperometric measurement for efficient process control of PAA concentration. The amperometric PAASensor is an on-line sensor that uses a membrane that allows the selective diffusion of PAA molecules to the amperometric sensor without cross sensitivity with hydrogen peroxide. However, it can present some interference with the dissolved matter for pesticides such as in the case of citrus and organic matter.

- **Test methods: PAASense** (Palintest, Water Analysis Technology, UK) is a simple, accurate, rapid and inexpensive measurement procedure based on a sophisticated electrochemical measurement technique with repeatable and reliable measurements, suitable for a wide range of produce water and PAA concentrations (**Figure 9**).

The technology of this sensor is driven by disposable single-use sensors which utilize chronoamperometry and electrochemistry to measure the electrical current generated by chemical reactions. Chronoamperometry involves applying a fixed voltage to an electrode and recording the resulting current over time. The magnitude of the current is proportional to the concentration of peroxyacetic acid in the test sample.

Figure 9. PAASense (Palintest, Water Analysis Technology, UK).

PAASense is recommended for calibration of amperometric probes and the control of the disinfection process as it quantifies the residual PAA concentration needed in the wash water.

3.5 Commercial products

Citrocide PC (Citrosol, https://www.citrosol.com) is a processing aid for the treatment of process water of citrus fruits, peppers, and tomatoes. Certified as a post-harvest product and it is compatible with organic production. In Spain, recommended levels are 0.4% for citrus and 0.2% for tomatoes for Citrocide Plus (15% PAA), and 0.6 % for citrus and peppers for Citrocide PC (5% PAA).

Part 4: Improve Performance of Water Treatment

4.1 Pre-shower

Much of the efforts must be directed towards developing approaches to diminish the concentration of organic matter in the process water. After cutting, a step to remove the exudates before produce is immersed in the washing tank is recommended to reduce the demand for the antimicrobial agent. A new processing design must include a pre-washing for shredded products to remove the cell exudates from the cut surfaces and significantly reduce the organic matter.

4.2 Water replenishment and wastewater treatment

Water replenishment helps to maintain the physicochemical quality of the process water, improving the efficacy of the process water treatments and reducing the potential accumulation of DBPs. However, the environmental impact and cost associated with higher water consumption and disposal is not desired. Another option to maintain the quality of wash water is the use of reconditioning treatments to improve water characteristics before reuse (e.g. membrane filtration systems to remove particulate material, physical disinfection treatments such as coagulation and flocculation).

For wastewater, the company AVANTech (Columbia, SC, USA) has developed the REFRESH™ system, that produces sanitized water removing bacteria, and can recycle >75% of the wash water. It reduces water costs, sewer charges, and energy needed for water chilling.

4.3 Raw material

The most significant factor that affects the microbiological quality of the final product are the microbial levels encountered on the incoming material. Process water treatments can inactivate microorganisms efficiently in the water, but their efficacy on the produce is limited.