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Microbial and chemical characterization of commercial washing lines of fresh produce highlights the need for process water control

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A B S T R A C T

Process wash water and washed products from three different fresh produce processing lines were characterized at commercial scale. Different physicochemical and microbiological characteristics of wash water were measured. Great variability between processing lines on the physicochemical quality of process wash water was observed, caused in part by the type of produce washed. The relationship between lower aerobic mesophilic bacteria and higher free chlorine (FC) concentrations in wash water was detected (Pearson's correlation coefficient (PCC) = −0.53). Independently of the FC concentration, most of the water samples (> 80%) showed presence of cultivable (limit of detection 1 CFU/100 mL), probably caused by the uncontrolled pH conditions. Higher values of FC and oxidation-reduction potential (ORP) in wash water were related to lower microbial load in washed produce (PCC = −0.82, and − 0.79, respectively). Higher concentration of chlorine was linked to a higher presence of disinfection by-products (DBPs) in the wash water, and washing in chlorinated water led to a significant increase in the concentration of DBPs in produce. However, the accumulation of trihalomethanes (THMs) in process wash water was not correlated with higher concentrations of these DBPs in produce.

Industrial relevance: The washing step of fresh produce processing lines is a critical process. The dose of disinfectants needs to be adequately optimized to avoid microbial contamination without generating the accumulation of disinfection by-products (DBPs). In this study, critical parameters that influence the efficacy of water disinfection and the occurrence of DBPs in fresh produce processing lines were identified under commercial conditions. The results evidenced that monitoring and control of pH play a critical role by maximizing the concentration of the most active form of chlorine in the water. The parameter UV254 measured on-line in the washing tank, can be suggested as a suitable indicator of the presence of organic matter in fresh produce wash water.

1. Introduction

The fresh produce industry demands high volumes of water to wash its products in a ratio of 10 L of water per kg of processed product, approximately. High quantities of wastewater are generated on a daily base (Manzocco et al., 2015). To reduce the high water demand, strategies to keep the chemical and microbiological quality of the process water, enabling its recycling, are needed (Casani, Rouhany, & Knøchel, 2005). Among the strategies available, water disinfection technologies are essential for keeping the microbiological quality of process wash water from the fresh produce industry (Gil, Selma, López-Gálvez, & Allende, 2009).

The presence of sanitizers in process wash water has limited efficacy regarding microbial reductions on fresh produce (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008; Ilic, Odomeru, & LeJeune, 2008). On the other hand, wash water disinfection is needed to avoid cross-contamination between contaminated and uncontaminated plant material, reducing the health risks associated with the presence of pathogenic bacteria in fresh-cut produce (Danyluk & Schaffner, 2011; Gombas et al., 2017; Maffei, Sant'Ana, Franco, & Schaffner, 2017).

To optimize wash water sanitation, it is necessary to ascertain the minimum disinfectant doses needed to avoid cross-contamination while minimizing the formation of DBPs (Gómez-López, Lannoo, Gil, & Allende, 2014; Zhou, Luo, Nou, & Millner, 2014). Sanitizer concentration in the process wash water should continually be monitored and controlled to guarantee the performance of the washing system. However, to assess the operational efficacy of a washing process is very difficult due to the constantly changing conditions of the different production lines (Warriner & Namvar, 2013), with production rates as low as 100 kg to up to 6 tons of fresh produce per hour. Furthermore,

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many different factors such as the configuration of the processing lines, the standard operating procedures (SOP) used to control the dosage of the sanitizing agent and the type of fresh produce processed among others vary between processing lines, companies, and regions.

Wash water quality parameters (e.g., chemical oxygen demand (COD)) are linked to chlorine demand and therefore with chlorine replenishment. Operative decisions can affect the antimicrobial efficacy of chlorine (e.g., target pH range in wash water) and the accumulation of DBPs (e.g., chemicals used to adjust pH). In the last years, a considerable effort has been made to establish the factors that affect the efficacy of process wash water sanitation during fresh produce processing. Major discussed research topics include: the chlorine demand during fresh produce washing (Chen & Hung, 2017; Toivonen & Lu, 2013; Weng et al., 2016), the minimum disinfectant concentrations needed to avoid cross-contamination with pathogenic microorganisms while minimizing formation of DBPs (Gil, Marín, Andujar, & Allende, 2016; Gómez-López et al., 2014; Zhou, Luo, Nou, Lyu, & Wang, 2015), and advances to monitor and control fresh produce washing operations (Azimi, Munther, Fakoorian, Nguyen, & Simon, 2017; Zhou et al., 2014).

The present study aimed to characterize the performance of three commercial fresh produce processing lines (baby leaves, fresh-cut lettuce, and shredded vegetables), to search for those critical water quality variables that allow the control of the washing process taking into account the microbiological and chemical safety of the fresh produce. Data obtained in commercial fresh produce processing lines were used to assess the relationships between the characteristics of the wash water and the presence of microorganisms and disinfection by-products in wash water and in produce.

2. Materials and methods

2.1. Plant material, processing lines and monitoring plan

Three different processing lines from a Spanish processor were assessed. The processing lines included baby leaves (mixes including red oak leaf lettuce, lamb's lettuce, rocket, red chard, Mizuna, red batavia, and green lollo lettuce), fresh-cut lettuce (romaine and frisée lettuce, in 3 cm shreds), and shredded vegetables (including carrot, cabbage, and fresh-cut tomato). Chlorine used for wash water sanitation was supplied in the form of calcium hypochlorite to keep the desired free chlorine residual in the process wash water, and in the form of sodium hypochlorite for the initial adjustment of free chlorine and also for sporadic application in moments of high chlorine demand. The processing plant was visited three times, one visit per line. Sampling started at the start of the washing process and it was monitored over 3–5 h depending on the production load of each line.

The washing lines comprised two separate tanks holding 3000 L of cold (3–4 °C) chlorinated tap water each (Fig. 1). Water, after a coarse filtration through a stainless still grid, recirculated from the second tank to the first tank, and the product moved along the washing tanks by means of the chlorinated tap water flow. After the second tank, the product was rinsed with showers of cold tap water. Rinsing water replenished the wash water that exited the tank with the produce. Calcium hypochlorite tablets were placed in small tanks adjoined to the washing tanks. Water flowed from the washing tank to the small tank and then returned to the washing tank at the point where the product was introduced. Water flow and recirculation ensured the constant entry and the proper mixing of disinfectant in the washing tank. Liquid sodium hypochlorite was added when needed directly at the produce entry area of the washing tanks. According to the processor, the targeted free chlorine (FC) range in the washing tanks was 40–60 mg/L, and the targeted pH range was between 6.5 and 7.5. pH was regulated using citric acid. During the sampling period, produce entered the washing area at an average rate of 250, 420 and 485 kg/h in the baby leaves, fresh-cut lettuce, and shredded vegetables lines, respectively. In the three lines, 10 L of water were consumed per each kg of produce that was processed during the sampling period. The design of the washing lines guarantees a minimum contact time of produce with chlorinated water of 30 s from the entry in the first tank to the rinsing showers.

2.2. Water sampling and analysis

Wash water samples (2 L) were taken in sterile containers following the scheme included in the Supplementary material (S1). Free chlorine (FC), total chlorine (TC), pH, temperature, oxidation-reduction potential (ORP), and electrical conductivity (EC) were measured every 15–20 min in water samples (100 mL) of both washing tanks except in the baby leaves line, in which only the first tank was sampled. FC and TC levels were determined by the DPD method (APHA, 1998) using the Spectroquant NOVA 60 photometer (Merck, Darmstadt, Germany) and the corresponding test kits. Combined chlorine (CC) values were calculated by subtracting the results of FC and TC measurements (Eq. (1)). Temperature, ORP, pH, and EC were measured using a portable multimeter sensION+ MM150 (Hach, Loveland, Colorado, USA).

$$\text{Combined chlorine}\left(\frac{\text{mg}}{\text{L}}\right) = \text{Total chlorine}\left(\frac{\text{mg}}{\text{L}}\right) - \text{Free chlorine}\left(\frac{\text{mg}}{\text{L}}\right) \quad (1)$$

Analyses of other physicochemical and microbiological parameters of the process wash water were performed in samples taken every hour, approximately. For these analyses, three bottles were filled (2 L) with water from the first washing tank of each of the processing lines. The samples were transported immediately to a laboratory located in the processing plant. In the laboratory, a volume of 1 L was transferred from each bottle into another sterile bottle containing enough (0.5–1.5 g) solid sodium thiosulphate pentahydrate (Scharlau, Barcelona, Spain) for the quenching of disinfectant residuals. The

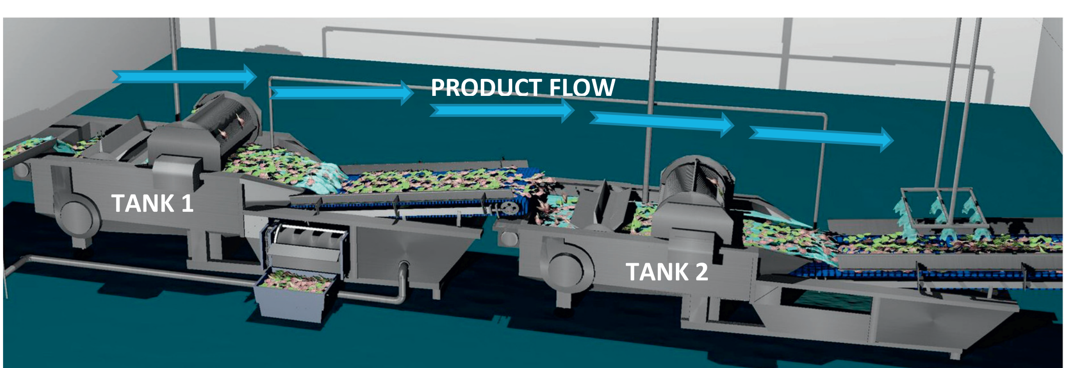

Fig. 1. Diagram of the washing operation in commercial fresh produce processing lines.

neutralized samples were used for the enumeration of microorganisms and the quantification of disinfection by-products (DBPs). All water analyses were performed in duplicate within the same day of sampling.

Non-neutralized water samples were transported to the CEBAS-CSIC lab in refrigerated conditions and used for the evaluation of physicochemical parameters the same day of sampling, including turbidity, total dissolved solids (TDS), absorbance at 254 nm (UV254) and chemical oxygen demand (COD). Turbidity was measured using a turbidimeter Turbiquant 3000 IR (Merck). TDS were analyzed using APHA 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 measured using a UV-VIS spectrophotometer (Jasco V-630, Tokyo, Japan) and 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).

Presence of cultivable aerobic mesophilic bacteria, 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 aerobic mesophilic count, 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. In addition, an enrichment step was used to assess the presence 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 the analysis of DBPs, neutralized water samples were transferred to fill an amber glass bottle to the top (approximately 120 mL). The bottle was tightly closed and kept under refrigeration until analysis. The concentration of chlorates in wash water was assessed in three samples each sampling time using a UPLC-MS as explained in Gil et al. (2016). The detection and quantification limits for chlorates in water were 0.0007 mg/L and 0.0024 mg/L, respectively. Trihalomethanes (THMs) were analyzed by GC–MS as explained in Gómez-López, Marín, Medina-Martínez, Gil, and Allende (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). The detection limits in μg/L were: 0.41, 0.76, 0.31 and 0.17 for trichloromethane, bromodichloromethane, dibromochloromethane, and tribromomethane, respectively.

2.3. Fresh produce sampling and analysis

Fresh produce samples were taken in triplicate at 4–6 time intervals and in three different sampling points including unwashed, washed and rinsed fresh produce. In the case of baby leaves and shredded vegetables, it was not possible to get samples at the first sampling time (time 0), immediately before the start of the processing line. Fresh produce samples (200 g each) were taken in sterile bags and directly transferred to the laboratory located in the factory. Each sample was individually centrifuged for 1 min using clean kitchen centrifuges. Centrifuges were disinfected with ethanol (70%) and rinsed with tap water between samples.

For the microbiological analyses, centrifuged fresh produce samples (25 g each) were transferred to sterile stomacher bags and mixed with 100 mL of BPW (2 g/L) supplemented with sodium thiosulphate (0.1 g/L) to quench disinfectant residuals. For the detection of cultivable aerobic mesophilic bacteria, total coliforms and *E. coli*, pour and surface plating of the homogenized samples were performed using the media and incubation conditions previously explained for process wash water analysis.

For the analysis of DBPs, centrifuged and non-neutralized fresh produce samples (30 g each) were taken and chopped during 10 s using a meat mincer (Moulinex A320, Moulinex, Ecully, France). The chopped samples were used for both chlorate and THM analyses. For chlorate analyses, 10 g of chopped fresh produce 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 explained before (Gil et al., 2016). The detection and quantification limits for chlorates in produce were the same as indicated for process wash water. For THMs analysis, an amount of 1 g of chopped produce sample was transferred to SPME vials, and samples were analyzed by GC–MS as explained above (Gómez-López et al., 2013). The detection and quantification limits for THMs in produce were the same as indicated for process wash water. Results were expressed in mg/kg FW for chlorates and in μg/kg FW for THMs.

2.4. Statistical analysis

Data on microbial populations were log-transformed. Graphs were made using Sigma Plot 12.0 Systat Software, Inc. (Addilink Software Scientific S.L., Barcelona, Spain). IBM SPSS statistics 24 was used for statistical analysis. Shapiro Wilk test and Levene's test were used to assessing normality and equality of variance, respectively. When normality could be assumed, *t*-tests were used to compare two treatments. Also assuming normality, One-way ANOVA was performed to compare more than two treatments, with Tukey's HSD or Dunnett's as post hoc tests depending on the homogeneity of the variances. When data was not following a normal distribution, nonparametric tests (Mann–Whitney U and Kruskal–Wallis) were applied to determine the differences between treatments. Pearson's correlation coefficient was calculated to evaluate the correlation between data. The calculation of the correlations was performed pooling all the data from the three lines. The calculation of the correlations for each of the production lines individually was not achieved due to the limited amount of data available. For the interpretation of results, it was considered that there was a correlation between two parameters when the correlation was significant at $\alpha = 0.01$. The strength of the correlation was assessed using the guide that Evans (1996) suggested for the absolute value of r: 0.00–0.19 “very weak”, 0.20–0.39 “weak”, 0.40–0.59 “moderate”, 0.60–0.79 “strong”, 0.80–1.0 “very strong”.

3. Results

3.1. Physicochemical characteristics of process wash water

Variation in FC throughout the sampling period was wider in the line of shredded vegetables, while it was relatively stable in the other two processing lines (baby leaves and fresh-cut lettuce) (Fig. 2A). Free chlorine was significantly different ($p < 0.05$) among processing lines, that can be ordered as shredded vegetables > fresh-cut lettuce > baby leaves from the highest to the lowest average free chlorine concentrations (Supplementary material: S2). There were also differences between tanks within the same processing line. Free chlorine was significantly higher ($p < 0.05$) in the first tank compared to the second one in the shredded vegetables and the fresh-cut lettuce lines. Regarding CC, it was significantly higher ($p < 0.05$) in the line of shredded vegetables compared with the other processing lines, and it was significantly higher ($p < 0.05$) in the first tank compared with the second in the shredded vegetable line (Fig. 2B).

The pH of the wash water was more or less stable throughout the

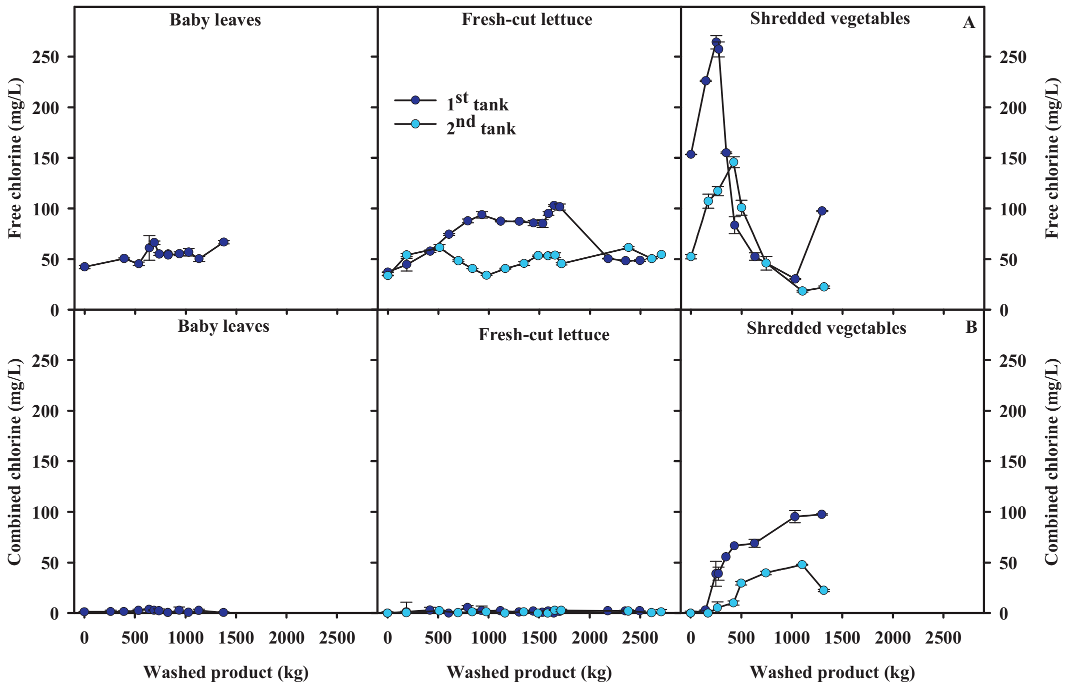

Fig. 2. Changes in the concentration of free chlorine (A) and combined chlorine (B) (mg/L) in the wash water from commercial fresh produce processing lines.

sampling period in the lines of baby leaves and fresh-cut lettuce, while it tended to increase in the shredded vegetables line (Fig. 3). Taking into account data from the washing tanks of all the processing lines, pH ranged between 4.2 and 8.3. The temperature of the wash water was well controlled and, therefore, it was quite stable in all the processing lines ranging between 4.0 and 6.5 °C (Supplementary material: S3). The oxidation-reduction potential of the wash water in the shredded vegetable line showed a trend to decrease throughout the sampling period, while it was more or less stable in baby leaves and fresh-cut lettuce (Supplementary material: S4). Taking into account data from all the processing lines and all the washing tanks, ORP ranged between 724 and 990 mV with no significant differences within the two washing tanks from the same line. There were significant differences in the EC between lines ($p < 0.05$) (shredded vegetables > fresh-cut lettuce > baby leaves). The electrical conductivity of the wash water was more or less stable throughout the sampling period in the baby leaves and fresh-cut lettuce lines. The shredded vegetables line showed an increase in EC throughout the sampling period, (Supplementary material: S5), and it registered the lowest (2.01 μS/cm, in the second washing area) and the highest (2460 μS/cm, in the first washing area) EC values.

Values of COD, UV254, turbidity, and TDS tended clearly to increase along the sampling period, more markedly in the case of shredded vegetables (Table 1). The order of the processing lines from higher to lower average values of COD, UV254 and TDS was shredded vegetables > fresh-cut lettuce > baby leaves. Regarding COD and TDS, differences between lines were significant ($p < 0.05$) in all cases. Maximum values of COD were 7110, 439 and 120 mg/L in the shredded vegetables, fresh-cut lettuce and baby leaves lines, respectively. Absorbance at 254 nm (UV254) was significantly higher ($p < 0.05$) in the shredded vegetables and fresh-cut lettuce lines compared with the baby leaves line. The highest values of UV254 were 0.09, 0.39, and 1.06 cm^{-1} in the baby leaves, fresh-cut lettuce and shredded vegetables lines, respectively. In the case of turbidity, the order of the processing lines from higher to lower average values was shredded vegetables > baby leaves > fresh-cut lettuce, although there were no significant differences between lines ($p > 0.05$). The maximum turbidity values were 295, 39 and 23 NTU in the lines of shredded vegetables, fresh-cut lettuce, and baby leaves, respectively. Average values of TDS were 7.5, 0.91 and 0.61 g/L for the shredded vegetables, fresh-cut lettuce, and baby leaves lines, respectively.

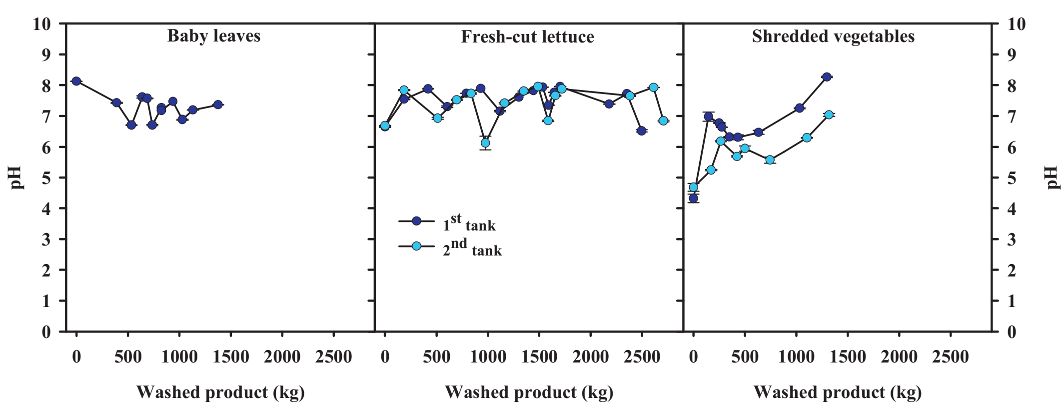

Fig. 3. Changes in the pH of the wash water from commercial fresh produce processing lines.

Table 1
Chemical oxygen demand (COD), absorbance at 254 nm (UV254), turbidity and total dissolved solids in the wash water of commercial fresh produce processing lines.

Processing line	Sampling time (h)	Washed product (kg)	COD (mg/L)	UV254 (cm^{-1})	Turbidity (NTU)	Total dissolved solids (g/L)
Baby leaves	0	0	< 25	0.06 ± 0.00	11 ± 3	0.29 ± 0.01
	1	390	34 ± 1	0.07 ± 0.00	21 ± 5	0.34 ± 0.00
	2	738	35 ± 1	0.08 ± 0.00	19 ± 4	0.36 ± 0.00
	3	938	80 ± 2	0.06 ± 0.00	24 ± 3	0.40 ± 0.05
	4	1030	74. ± 0	0.06 ± 0.00	20 ± 2	0.37 ± 0.05
	5	1377	112 ± 11	0.09 ± 0.00	38 ± 2	0.51 ± 0.14
Fresh-cut lettuce	0	0	72 ± 5	0.03 ± 0.00	4 ± 0	0.55 ± 0.05
	1	605	302 ± 8	0.14 ± 0.00	16 ± 0	0.74 ± 0.02
	2	1116	297 ± 0	0.15 ± 0.00	19 ± 3	0.74 ± 0.03
	3	1590	305 ± 3	0.14 ± 0.00	15 ± 0	0.78 ± 0.01
	4	1702	375 ± 89	0.11 ± 0.04	11 ± 0	0.86 ± 0.07
	5	2352	298 ± 1	0.39 ± 0.00	21 ± 3	0.75 ± 0.02
Shredded vegetables	0	0	448 ± 110	0.03 ± 0.00	1 ± 0	1.20 ± 0.10
	1	275	1012 ± 14	0.26 ± 0.00	16 ± 0	2.00 ± 0.01
	2	632	5197 ± 3	0.89 ± 0.02	215 ± 13	5.60 ± 0.06
	3	1297	7092 ± 24	1.04 ± 0.02	287 ± 11	7.20 ± 0.44

Data are the mean ± standard deviation of the two samples taken each sampling time. Statistical differences in the value of the parameters between sampling times in a line could not be calculated due to the limited amount of data available (two data per sampling time).

3.2. Microbiological quality of wash water and fresh-cut products

The population of cultivable aerobic mesophilic bacteria present in the wash water was very variable, ranging from below the limit of detection (1 CFU/100 mL) to 4.86 log CFU/100 mL (Table 2). However, in most of the cases, the microbial load of process wash water was below 100 CFU/100 mL. In wash water of baby leaves and fresh-cut lettuce, 100% and 83.3% of the samples were positive for the presence of aerobic mesophilic bacteria, respectively. In contrast, only 25% of the samples of shredded vegetables were positive for the presence of aerobic mesophilic microorganisms. Prevalence and population of total coliforms in wash water were lower in comparison with that of aerobic mesophilic bacteria, with a maximum concentration lower than 0.5 log CFU/100 mL in the wash water of baby leaves (Table 2). The proportion of positive samples for total coliforms was 66.6%, 33.3%, and 0% in the wash water of baby leaves, fresh-cut lettuce and shredded vegetables, respectively. *E. coli* was not detected in any of the wash water samples analyzed after filtration (limit of detection 1 cfu/100 mL) or enrichment (absence in 50 mL) (Table 2).

Table 2
Microbiological characteristics of the wash water from the first washing tank of commercial fresh produce processing lines.

Processing line	Sampling Time (h)	Washed product (kg)	Aerobic mesophilic bacteria (log CFU/100 mL)	Total coliforms (log CFU/100 mL)	*E. coli*
Baby leaves	0	0	3.69 ± 0.11	0.15 ± 0.21	Absence*
	1	390	3.24 ± 0.18	0.39 ± 0.12	Absence
	2	738	1.66 ± 0.05	0.35 ± 0.49	Absence
	3	938	3.59 ± 0.09	< 0	Absence
	4	1030	0.83 ± 0.18	< 0	Absence
	5	1377	0.99 ± 0.12	0.24 ± 0.34	Absence
Fresh-cut lettuce	0	0	2.24 ± 0.34	< 0	Absence
	1	605	1.03 ± 0.47	0.15 ± 0.21	Absence
	2	1116	0.66 ± 0.26	< 0	Absence
	3	1590	0.59 ± 0.16	< 0	Absence
	4	1702	< 0	< 0	Absence
	5	2352	1.88 ± 0.85	0.15 ± 0.21	Absence
Shredded vegetables	0	0	< 0	< 0	Absence
	1	275	< 0	< 1	Absence
	2	632	4.86 ± 0.66	< 1	Absence
	3	1297	< 0	< 1	Absence

Data are the mean ± standard deviation of the two samples taken each sampling time. *Negative result by filtration of 100 mL, and by plating an enrichment (50 mL of wash water + 50 mL buffered peptone water 40 g/L, 24 h at 37 °C). Statistical differences in the value of the parameters between sampling times in a line could not be calculated due to the limited amount of data available (two data per sampling time).

For unwashed samples, fresh-cut lettuce had significantly higher mesophilic and coliform counts (7.5 ± 0.19 and 5.3 ± 0.3 log CFU/g, respectively) compared to that of baby leaves and shredded vegetables ($p < 0.05$). The effect of washing and rinsing steps on the cell densities in the produce varied within the processing lines. However, in all cases, counts of aerobic mesophilic bacteria and total coliforms were significantly higher in unwashed produce compared to washed and rinsed produce ($p < 0.05$) (Table 3). The washing step in chlorinated water reduced mesophilic counts by 0.44 ± 0.35, 2.22 ± 0.47, and 1.46 ± 0.43 log units in the case of baby leaves, fresh-cut lettuce and shredded vegetables, respectively. On the other hand, the population of total coliforms was reduced by 0.83 ± 0.36, 1.73 ± 0.31, and 1.24 ± 1.13 log units, in baby leaves, fresh-cut lettuce, and shredded vegetables, respectively. Additional logarithmic reductions in aerobic mesophilic bacteria achieved by the rinsing step were 0.43 ± 0.33, 0.05 ± 0.44, and 0.15 ± 1.08 in the case of the baby leaves, fresh-cut lettuce, and shredded vegetables, respectively. The population of total coliforms was reduced in the rinsing step by 1.22 ± 0.85, 0.05 ± 0.53, and 0.07 ± 0.79 log units, in baby leaves, fresh-cut lettuce, and shredded vegetables, respectively. *E. coli* levels in produce samples were always below the limit of detection (5 CFU/g).

3.3. Disinfection by-products in wash water and fresh-cut products

Significant differences in the concentration of chlorates in water between lines were found ($p < 0.05$). The concentration of chlorates in the wash water was quite stable throughout the sampling period in the baby leaves and fresh-cut lettuce lines, while it clearly increased in the shredded vegetables line (Fig. 4A). The maximum and minimum concentrations of chlorates measured were 47.4 mg/L in the shredded vegetables line and 0.44 mg/L in the baby leaves line, respectively. The average concentration of chlorates in the wash water from the first washing tank was 1.2 ± 0.5, 2.7 ± 0.5, and 29.1 ± 11.9 mg/L in the baby leaves, fresh-cut lettuce and shredded vegetables lines, respectively.

Table 4 shows the concentration of chlorates and trihalomethanes (μg/kg) on fresh-produce from the different processing lines at different sampling times. Chlorate was below the limit of detection in all the unwashed fresh-cut lettuce samples. In baby leaves and shredded vegetables, some unwashed produce samples were below the limit of detection, while others showed detectable concentrations (maximum 77.1 μg/kg in baby leaves). In general, washing in chlorinated water led

Table 3
Population of aerobic mesophilic bacteria and total coliforms (log CFU/g) in produce from commercial fresh produce processing lines.

Processing line	Sampling time (h)	Processed product (kg)	Aerobic mesophilic bacteria			Total coliforms		
			Unwashed	Washed	Rinsed	Unwashed	Washed	Rinsed
Baby leaves	0	0	NA	NA	NA	NA	NA	NA
	1	390	6.14 ± 0.02	5.72 ± 0.33	5.66 ± 0.30 ns	4.05 ± 0.40a	3.68 ± 0.69a	2.12 ± 0.50b
	2	738	6.14 ± 0.33a	6.08 ± 0.07a	5.23 ± 0.41b	4.12 ± 0.93a	3.21 ± 1.67ab	1.20 ± 0.63b
	3	938	6.26 ± 0.13a	5.89 ± 0.26a	5.21 ± 0.17b	4.18 ± 0.12a	3.42 ± 0.39ab	2.09 ± 0.99b
	4	1030	NA	5.85 ± 1.09	5.50 ± 1.26 ns	NA	2.79 ± 0.59	1.97 ± 1.37 ns
	5	1377	5.67 ± 0.15a	4.76 ± 0.55ab	4.57 ± 0.29b	3.56 ± 0.03a	2.31 ± 0.71b	2.41 ± 0.72b
Fresh-cut lettuce	0	0	7.36 ± 0.28a	4.88 ± 0.18c	5.51 ± 0.09b	5.31 ± 0.21a	3.65 ± 0.68b	3.92 ± 0.82ab
	1	605	7.73 ± 0.27a	4.96 ± 0.48b	5.24 ± 0.54b	5.59 ± 0.29a	3.59 ± 0.33b	4.01 ± 0.81ab
	2	1116	7.33 ± 0.58a	4.82 ± 0.49b	5.09 ± 0.11b	5.19 ± 0.33a	3.18 ± 0.39b	3.72 ± 0.17b
	3	1590	7.29 ± 0.35a	5.17 ± 0.42b	4.91 ± 0.39b	4.98 ± 0.37a	3.73 ± 0.82ab	3.30 ± 0.20b
	4	1702	7.37 ± 0.13a	5.07 ± 0.39b	5.42 ± 0.65b	5.51 ± 0.07a	3.55 ± 0.33b	3.95 ± 0.59b
	5	2352	7.54 ± 0.17a	5.99 ± 0.14b	5.87 ± 0.11b	5.63 ± 0.31a	4.12 ± 0.83b	3.55 ± 0.24b
Shredded vegetables	0	0	NA	NA	NA	NA	NA	NA
	1	275	3.69 ± 0.46	1.79 ± 0.17	2.93 ± 2.13 ns	1.69 ± 0.0	1.69 ± 0.0	1.79 ± 0.17 ns
	2	1031	6.15 ± 0.06	5.12 ± 0.85	4.69 ± 0.71 ns	5.28 ± 0.14	3.77 ± 0.56	4.09 ± 0.89 ns
	3	1297	6.05 ± 0.15a	4.60 ± 0.37b	4.74 ± 0.49b	4.79 ± 0.18a	2.58 ± 0.57b	3.11 ± 0.43b

NA: not analyzed. Data are the mean ± standard deviation of three samples of produce taken each sampling time. Different letters in the same row indicate significant differences ($p < 0.05$) in the population of microorganisms between different conditions of the product (unwashed, washed, rinsed) for a specific sampling time. ns: not significant.

to a significant increase in the concentration of chlorates in fresh produce. Rinsing with tap water significantly reduced the concentration of chlorates in all the cases, except for one sample of shredded vegetables, where chlorates were not detected (Table 4).

Regarding THMs, the concentration in the wash water was quite stable throughout the sampling period in the baby leaves and fresh-cut lettuce lines, while it decreased in the shredded vegetables line (Fig. 4B). In wash water, the maximum concentration of THMs was 4227 μg/L in shredded vegetables, and the minimum was 1.9 μg/L in fresh-cut lettuce. The average concentration of THMs in the wash water from the first washing tank was 34.1 ± 27.4, 54.4 ± 24.9, and 2635.2 ± 824.9 μg/L in baby leaves, fresh-cut lettuce and shredded products, respectively. The line of shredded vegetables had a significantly higher ($p < 0.05$) concentration of THMs compared with the other two lines.

The concentration of THMs was below the limit of detection in all unwashed produce samples, and it was significantly higher ($p < 0.05$) in the washed products compared with unwashed ones (Table 4). Washed produce samples had an average THMs concentration of 19.9 ± 8.5, 16.2 ± 11.5 and 19.4 ± 20.2 μg/kg in baby leaves, fresh-cut lettuce and shredded vegetables, respectively. The rinsing step reduced the concentration of THMs to a final mean concentration of 19.4 ± 12.8, 10.1 ± 6.5, and 10.6 ± 9.3 μg/kg in baby leaves, fresh-cut lettuce and shredded vegetables, respectively. However, the observed reductions were significant in fresh-cut lettuce ($p < 0.05$), but not in baby leaves and shredded vegetables ($p > 0.05$).

3.4. Correlation between microbiological and physicochemical parameters

The value of the Pearson's correlation coefficient (PCC) and the

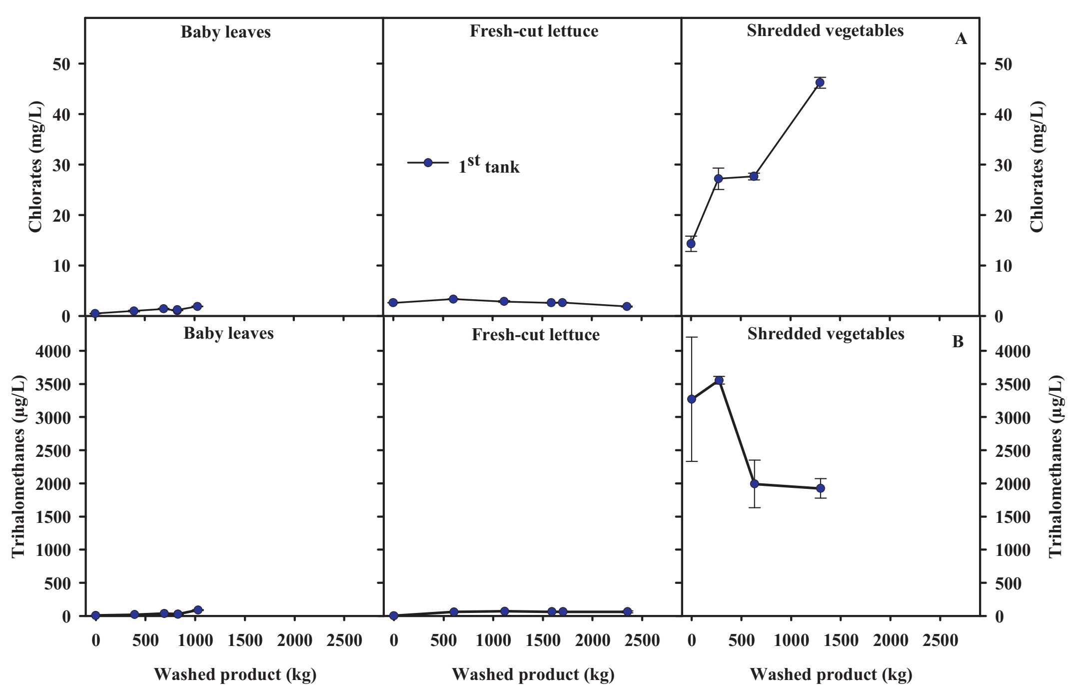

Fig. 4. Changes in the concentration of chlorates (mg/L) (A) and trihalomethanes (μg/L) (B) in the wash water from commercial fresh produce processing lines.

Table 4
Concentration of chlorates and trihalomethanes (μg/kg) on fresh-produce from commercial processing lines.

Processing line	Sampling time (h)	Processed product (kg)	Chlorates			Trihalomethanes		
			Unwashed	Washed	Rinsed	Unwashed	Washed	Rinsed
Baby leaves	0	0	NA	NA	NA	NA	NA	NA
	1	390	72.8 ± 3.7b	180.6 ± 26.3a	103.1 ± 8.2b	NDb	6.3 ± 2.9a	4.2 ± 0.9ab
	2	738	NDc	144.4 ± 13.9a	33.8 ± 2.4b	NDb	23.9 ± 5.2a	21.3 ± 2.6a
	3	938	25.7 ± 4.5c	213.7 ± 25.9a	114.2 ± 20.4b	NDc	27.6 ± 5.2b	40.6 ± 5.9a
	4	1030	23.5 ± 8.9b	149.4 ± 29.4a	54.1 ± 14.7b	NDc	18.9 ± 1.9a	13.5 ± 2.5b
	5	1377	NDb	299.5 ± 82.8a	238.8 ± 37.9a	NDb	22.7 ± 6.1a	17.1 ± 2.2a
Fresh-cut lettuce	0	0	NDc	233.7 ± 43.3a	125.9 ± 31.3b	NDb	12.0 ± 3.2a	3.7 ± 0.9b
	1	605	NDb	206.8 ± 34.5a	150.3 ± 23.8a	NDb	8.9 ± 0.6a	7.9 ± 1.5a
	2	1116	NDc	165.8 ± 16.9a	42.0 ± 22.3b	NDb	16.9 ± 2.2a	21.6 ± 3.7a
	3	1590	NDc	101.8 ± 29.3a	66.4 ± 5.4b	NDc	39.9 ± 5.9a	13.9 ± 2.1b
	4	1702	NDb	113.4 ± 33.4a	76.1 ± 14.9a	NDb	10.9 ± 2.8a	8.4 ± 2.9a
	5	2352	NDb	264.4 ± 87.4a	186.4 ± 5.4a	NDc	8.8 ± 1.8a	5.1 ± 1.1b
Shredded vegetables	0	0	NA	NA	NA	NA	NA	NA
	1	275	0.1 ± 0.1	ND	NDns	NDb	3.7 ± 1.4a	3.5 ± 1.3a
	2	1031	4.7 ± 0.3c	133.4 ± 3.2a	79.2 ± 11.8b	NDc	44.4 ± 14.1a	22.3 ± 5.7b
	3	1297	3.8 ± 0.5c	101.2 ± 11.2a	43.3 ± 9.7b	NDb	10.1 ± 2.8a	5.9 ± 0.6a

NA: not analyzed. ND: not detected. Data are the mean ± standard deviation of the three samples taken each sampling time. Different letters in the same row indicate significant differences ($p < 0.05$) in the concentration of DBPs between different conditions of the product (unwashed, washed, rinsed) for a specific sampling time. ns: not significant.

number of data used to perform the correlation analysis are shown in parentheses.

3.4.1. Microbiological parameters

Regarding correlations of the population of microorganisms with other parameters measured in wash water from the first washing tank, log counts of mesophilic bacteria showed moderate negative correlation with FC (PCC = −0.53), while the population of total coliforms did not show correlation with any of the parameters measured.

Correlation between characteristics of washed produce and those of wash water from the first washing tank was assessed. The population of aerobic mesophilic bacteria in washed produce showed moderate positive correlation with the population of mesophilic bacteria in water (0.42, 39), strong negative correlation with ORP (−0.79, 42), and very strong negative correlation with the concentration of FC (−0.82, 42), and TC (−0.81, 42). The population of total coliforms in washed produce showed moderate negative correlation with ORP (−0.40, 42), FC (−0.48, 42), and TC (−0.47, 42). The correlation of coliforms in washed produce with coliforms load in wash water was not significant due to the low population found in water. However, the reductions in the microbial load of produce obtained by washing did not show correlation with physicochemical characteristics of wash water. The reductions in the population of total coliforms in produce showed very strong positive correlation with the total coliforms load of the unwashed plant material (0.81, 13).

Correlation between the characteristics of washed produce (taken from the second washing tank, before rinsing), and the characteristics of wash water from the second washing tank was also assessed. The population of aerobic mesophilic bacteria in washed produce showed strong positive correlation with pH (0.72, 27), strong negative correlation with FC (−0.79, 27), and very strong negative correlation with ORP (−0.88, 27), and TC (−0.86, 27). The population of total coliforms in produce showed strong negative correlation with ORP (−0.68, 27) and FC (−0.67, 27) of the wash water from the second tank.

3.4.2. Physicochemical parameters

With respect to the correlations of the concentration of DBPs with other parameters measured in wash water from the first washing tank, chlorates showed moderate positive correlation with FC (0.48), strong positive correlation with TC (0.79), and THMs (0.78), and very strong positive correlation with CC (0.94), EC (0.96), turbidity (0.83), TDS (0.92), UV254 (0.84), and COD (0.89). THMs showed moderate positive correlation with CC (0.57), TDS (0.55), and COD (0.49), strong positive correlation with EC (0.68) and FC (0.78), and very strong positive correlation with ORP (0.80) and TC (0.87). Free chlorine, apart from its correlations with mesophilic bacteria, THMs, and chlorates, showed strong positive correlation with ORP (0.60), and very strong positive correlation with TC (0.94). Combined chlorine (CC), COD, UV254, EC, turbidity, and TDS, correlated very strongly (> 0.80). Values of pH showed strong negative correlation with ORP (−0.76).

Regarding the correlation between water parameters from the second washing tank, in general, the trends observed were similar to those detected in the first washing tank. For example, FC showed a strong correlation with ORP (0.68), CC showed very strong correlation with EC (0.97), and pH showed very strong negative correlation with ORP (−0.91).

The concentration of THMs and chlorates in washed produce did not show any significant correlation with the water quality parameters measured in both first and second washing tank.

4. Discussion

The different types of fresh produce that are washed lead to differences in the physicochemical characteristics of the wash water in the processing lines. In our study, COD, TDS, and CC were significantly higher in the shredded vegetables line compared with the other two lines. Accordingly, in the study of Luo et al. (2018), differences between lines for the same parameters were found. In particular, COD, TDS, and CC were significantly higher in diced cabbage wash water when compared with chopped romaine lettuce and shredded iceberg lettuce. A constant increase in turbidity, COD, TDS, and CC during the working day in the wash water of the different processing lines was expected in agreement with previous studies in processing plants (Luo et al., 2018). In our study, this constant increase was observed in the line of shredded vegetables, but not in the lines of baby leaves and fresh-cut lettuce. One reason that could explain these differences could be the lower production of the processing lines characterized in our study. Luo et al. (2018) analyzed chopped romaine lettuce, shredded iceberg lettuce, and diced cabbage in lines with a product flow rate of 1560, 3660, and 2240 kg/h, respectively. In our study, baby leaves, fresh-cut lettuce, and shredded vegetables lines had a product flow rate of 258, 422, and 485 kg/h, respectively. These and other differences such as the ratio of the volume of water used per kg of produce, the frequency of water replenishment, and the washing tank design, highlight the variability between companies in the configuration and operation of the processing lines that leads to differences in wash water quality parameters.

The results obtained suggest that UV254 and turbidity, parameters that could be measured on-line in a washing tank, would be suitable indicators of the presence of organic matter in fresh produce wash water, as they behave in the same way as COD. Accordingly, Chen and Hung (2016, 2017), observed a strong correlation between UV254 and chlorine demand, and moderate correlation between turbidity and chlorine demand in simulated fresh produce wash water. Correlation between COD and turbidity, and between COD and UV254 in fresh-cut produce wash water has also been observed before at lab and pilot scale (Luo et al., 2012; Van Haute, Sampers, Holvoet, & Uyttendaele, 2013). Processing companies could use such parameters to obtain real-time data on the quantity of organic matter in the wash water and take operational decisions according to the results obtained (Zhou et al., 2014).

In processing lines with sequential double wash area configuration, the first wash area receives unwashed produce that releases exudates and debris. Therefore, the second wash area receives a cleaner product that introduces less organic matter in the wash water. However, in our study, a direct comparison could not be established because not all the water quality parameters were measured in both washing areas. However, FC, TC, pH, ORP, temperature, and EC were measured in both washing areas in our study, and in some cases, significant differences were detected. FC and CC, for example, were higher in the first wash area compared with the second one in shredded vegetables. The higher concentration of FC could be explained by a higher addition of sanitizer to cope with the higher demand of the water from the first washing area. The higher concentration of CC in the first washing area would have been caused by a higher concentration of organic matter due to the entrance of unwashed product combined with the higher amount of chlorine added to keep a higher concentration of FC. As observed before by Luo et al. (2018), in our study, more extensive fluctuations in FC and more rapid increase in CC were detected in the first washing area, that could be explained by the more abrupt changes in the concentration of chlorine demanding compounds caused by the entrance of unwashed product.

The concentration of the disinfectant, temperature, pH, and the presence of organic matter are the main factors that affect the inactivation of microorganisms in wash water using chlorine as the disinfectant (Suslow, 2001). Accordingly, in our study, the population of aerobic mesophilic bacteria in the water from the first washing area showed a negative correlation with the FC concentration measured in the water. Previously, Luo et al. (2018) also observed the relationship between the concentration of FC and the presence of bacteria in wash water at commercial scale. In their study, samples with FC concentrations higher than 20 mg/L showed bacterial counts lower than 1.7 log CFU/100 mL, while in our study, 35% of the samples have higher microbial load than the above level even when free chlorine concentrations were always above 20 mg/L. However, in most of the cases (69% of the samples), microbial load in process wash water, were very low (below 2 log CFU/100 mL) and they could be related to the entry of new product into the tank without enough contact time to inactivate the newly introduced microorganisms. In the case of the process wash water of the shredded vegetable line, only 25% of the samples were positive for the presence of microorganisms (limit of detection 1 CFU/100 mL). The most probable reason for the limited antimicrobial action when compared with previous results would be the high pH values of the wash water observed in our study (above 6.5 in most cases). pH showed a negative correlation with ORP, indicating a lower oxidant potential of the wash water when the pH was higher. Under the pH conditions observed in the processing lines surveyed in the present study, the fraction of chlorine that was in its most efficient form (hypochlorous acid, HClO) was minimal. The concentration of the most active form of chlorine in water highly depends on the temperature and the pH of the water (Randtke, 2010). To maximize the presence of hypochlorous acid, a pH below 6.5 should be maintained (Luo et al., 2018). Taking into account the pH, in the first washing tank the percentage of chlorine that was in the form of hypochlorous acid, the most active against microorganisms, was 75%, 65% and 95% approximately in the baby leaves, fresh-cut lettuce and shredded vegetable lines, respectively (Randtke, 2010).

Holvoet, Jacxsens, Sampers, and Uyttendaele (2012) surveyed wash water without any disinfectant from Belgian fresh-cut produce processing companies. In their study the total psychrotrophic aerobic counts detected in water ranged between < 2 and 9 log CFU/100 mL, total coliforms counts ranged between < 0 and 6.4 log CFU/100 mL, and the fecal indicator *E. coli* reached levels up to 5 log CFU/100 mL. In our study, in a processing plant that uses chlorine to disinfect the wash water, the maximum population of aerobic mesophilic bacteria and total coliforms was much lower (4.9 log and 0.39 log CFU/100 mL, respectively), and *E. coli* was not detected in water samples. The effect of the disinfectant was even more evident in the study of Luo et al. (2018), who observed very few wash water samples positive for aerobic mesophilic bacteria when the FC concentration was above 10 mg/L at pH = 5. In the processing lines analyzed in the present study, keeping a pH value below 6.5 combined with a free chlorine concentration in the range 15–20 mg/L could lead to the same or better results regarding the microbiological quality of water.

In the study of Maffei, Alvarenga, Sant'Ana, and Franco (2016), no linear correlation was found between the population of microorganisms in the wash water samples and those detected in the washed product. In our study, a moderate positive correlation could be found between counts of aerobic mesophilic bacteria in wash water and the washed product. On the contrary, Barrera, Blenkinsop, and Warriner (2012) concluded that the log reduction on produce was independent of the bacterial load of the wash water.

The acid used for pH adjustment can influence the presence of DBPs. In lab-scale experiments, Fan and Sokorai (2015) observed that the citric acid used to adjust pH in chlorinated wash water contributes to the formation of THMs in the product. In the processing plant that was surveyed in our study, citric acid was used to adjust the pH of the wash water. Substitution of citric acid for other acids (e.g., phosphoric acid) could help in reducing the amount of DBPs. Fan and Sokorai (2015) also observed that the formation of THMs depends on the type of vegetable. In their study, fewer THMs were formed in onion wash water although this water showed higher COD and turbidity compared with lettuce wash water. COD and turbidity are parameters linked to the concentration of organic matter in water, that is a critical factor in the formation of THMs in chlorinated process wash water (Gómez-López et al., 2013). The present study confirmed at the industrial level, previously obtained results showing that the concentration of THMs in water correlated with the concentration of chlorine (FC, TC, CC) and with parameters linked to the concentration of organic matter such as TDS, COD, and EC. The concentration of THMs was higher in the line of shredded vegetables compared with the other two lines. By previous findings (López-Gálvez et al., 2010), the higher chlorine and organic matter concentrations measured in the shredded vegetables wash water would have contributed to the higher concentration of THMs observed in this line compared with the other two lines. The concentration of chlorates in wash water correlated with different physicochemical parameters, namely COD, UV254, EC, turbidity, TDS, FC, TC, and CC. Chlorates present in the wash water probably proceed from the calcium hypochlorite and the sodium hypochlorite used as sanitizers. Therefore, when more organic matter is present in the wash water such as in the line of shredded vegetables, higher chlorine demand leads to the addition of a higher amount of chlorine to maintain the optimal operational conditions for water sanitization and more chlorates are introduced and accumulated in the process wash water.

Several lab-scale research studies evidenced that washing of fresh produce with sanitizers or clean water usually leads to microbial reductions of about 1 to 2 logs in produce, mostly because of the physical action of the water on the produce surface (Gil et al., 2009). The present study corroborates that moderate reductions (1–2 logs) can be found in a commercial processing line. Furthermore, the effect of the sanitizer was noticeable also in the negative correlation detected between the produce microbial load and the values of FC and ORP measured in the wash water. The water pH correlated positively with the microbial load of produce, highlighting the importance of pH regulation to maximize the efficacy of chlorine. Regarding the effect of the microbial load of wash water on the microbial contamination of produce, a moderate positive correlation between the population of aerobic mesophilic bacteria in wash water and produce was observed.

Previous lab-scale studies demonstrated a correlation between the concentration of chlorates in process wash water and produce (Gil et al., 2016). However, in the present study at industrial scale, this correlation could not be corroborated and only in the case of THMs, a weak relationship between the concentration of THMs in water and produce found at lab-scale studies has been confirmed at industrial scale (Gómez-López et al., 2013). By previous results (López-Gálvez et al., 2010), the rinsing step reduced to some extent the concentration of DBPs in produce.

5. Conclusions

The physicochemical quality of process wash water in a commercial fresh-cut produce production plant varied between processing lines. This variability could be explained by differences in the types of washed produce, but also by differences in the operational efficacy of the washing systems. Suitable parameters (turbidity, UV254) for real-time monitoring of the organic matter concentration in wash water were identified. Links between a lower microbial load of wash water and higher free chlorine concentration measured in the same wash water were detected. Furthermore, higher chlorine and ORP values in wash water were linked to lower microbial load in washed fresh produce. The results obtained highlight the usefulness of the use of sanitizers in fresh produce washing operations. However, higher concentrations of chlorine in water led to higher concentrations of DBPs in the wash water, and washing in chlorinated water led to significant increases in the concentration of DBPs present in produce. The findings obtained in this study supported previous results regarding the microbial reductions obtained at lab-scale research studies, but they also disproved former beliefs associated with the formation and accumulation of DBPs in fresh produce. In general, the free chlorine and pH regulations were not optimal, reducing the efficacy of the disinfectant and provoking the accumulation of DBPs. These results highlight the need to optimize the operation of fresh produce processing lines at commercial scale to reduce microbial and chemical risks linked to these types of products.

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