



Research Paper

Depletion of Free Chlorine and Generation of Trichloromethane in the Presence of pH Control Agents in Chlorinated Water at pH 6.5

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ABSTRACT

Chlorine is commonly used by the fresh produce industry to sanitize water and minimize pathogen cross-contamination during handling. The pH of chlorinated water is often reduced to values of pH 6–7, most commonly with citric acid to stabilize the active antimicrobial, hypochlorous acid (a form of free chlorine). Previous studies have demonstrated that citric acid reacts with chlorine to form trichloromethane, a major chlorine by-product in water and a potential human carcinogen. However, it is unclear if other pH control agents could be used in the place of citric acid to minimize the formation of trichloromethane. The objective of the present study was to determine the reactivity of organic and inorganic pH control agents, with chlorine, to generate trichloromethane. Free chlorine (~100 mg/L) was mixed with 10 mM of each of twelve organic acids and two inorganic pH control agents (i.e., sodium acid sulfate and phosphoric acid) to effect a pH level of 6.5. Free chlorine and trichloromethane levels were measured over 3 h at 3 and 22°C. Results demonstrated that ascorbic acid, dehydroascorbic acid, citric acid, and malic acid rapidly depleted free chlorine concentrations at both 22°C and 3°C, while tartaric acid and lactic acid decreased chlorine concentrations more slowly. Other pH control agents did not significantly reduce free chlorine either at 22 or 3°C. Citric acid led to the generation of significantly higher concentrations of trichloromethane than did other acids. Chloroacetone was also found in chlorinated water in the presence of citric acid and ascorbic acid. Taking buffering capacity and pKa values into account, phosphoric acid and some organic acids may be used to replace citric acid as pH control agents in chlorinated water for washing fresh produce, to stabilize free chlorine level and reduce the generation of trichloromethane.

Water washing is commonly utilized by the fresh produce industry to remove dirt and debris from produce surfaces, to cool the product, and to transport fresh-cut produce items during processing (López-Gálvez, Tudela, Allende, & Gil, 2019). To sanitize these waters, so as to minimize pathogen cross-contamination, sanitizers are often added to wash and rinse waters. Among the sanitizers, chlorine (sodium hypochlorite or NaOCl) is most commonly used by the industry, due to its availability, ease of use, and low cost (Juliana Rodrigues Gadelha et al., 2019). Free chlorine concentrations of 50–200 mg/L have often been used in fresh produce wash waters, although it has been recently demonstrated that maintaining free chlorine levels of 10–20 mg/L in wash waters is sufficient for many produce items (Gombas et al., 2017; Luo et al., 2018).

The antimicrobial efficacy of chlorine as a water disinfectant and a sanitizer is influenced by its concentration, as well as the temperature, and pH (Chen & Hung, 2017). The reactivity and efficacy of chlorine increase with increasing temperatures. Depending on the pH of the

chlorine (NaOCl) solution, the following three forms of chlorine may exist in water: (1) the hypochlorite ion (ClO⁻), (2) the chlorine diatom (Cl₂), and (3) hypochlorous acid (HOCl) (Ghernaout, 2017). Hypochlorous acid, the most effective biocidal form of chlorine, is abundant at pH levels of 5–7, while lower pH values may lead to the generation of chlorine gas. Therefore, the pH of chlorine solutions is often maintained close to 6.5, to generate a relatively high concentration of hypochlorous acid, while minimizing outgassing and generation of strong odors (Suslow, 1997). Citric acid is most commonly used by the produce industry to adjust and maintain the pH of chlorinated water (Herd & Feng, 2009; Marín et al., 2020), probably due to its high pKa (pKa3 = 6.4), low cost, ease of handling, and high water solubility (Lambros, Tran, Fei, & Nicolaou, 2022). The pKa values of most other organic acids are lower than 5, which do not have good buffering capacity in the pH range of 6–7.

One of the disadvantages of using chlorine as a sanitizer is the generation of potentially harmful chlorine by-products (Richardson,

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Plewa, Wagner, Schoeny, & DeMarini, 2007). The reaction of chlorine with organic materials (originating from produce) in water leads to the production of these disinfectant by-products. Trichloromethane is the major chlorine by-product generated in fresh produce wash waters (Gómez-López, Marín, Medina-Martínez, Gil, & Allende, 2013). The EPA's limit for total trihalomethanes, including trichloromethane, is 0.08 mg/L in drinking water because trichloromethane and other known chlorine by-products are potential human carcinogens and can increase the risk of liver, kidney, or central nervous system damage (EPA, 2022; National Toxicology Program, 2022).

Our previously published results demonstrated that citric acid readily reacts with chlorine, forming higher concentrations of chlorine by-products than those formed by chlorine reacting with organic materials (chemical oxygen demand value of 3,070 mg/L) from fresh produce (Fan & Sokorai, 2015). Similar results have also been reported by other researchers (Marín et al., 2020; Tudela et al., 2019). The high concentrations of trichloromethane in wash water may lead to the retention of these by-products in fresh-cut produce. Chlorine by-products can be absorbed (or attached) to the cut surface of fresh produce, particularly when the product is not further rinsed with chlorine-free water (Nitsopoulos et al., 2014). In one study, up to 1.13 mg/g of trihalomethanes were detected in ready-to-eat vegetables from retailers and processors in Italy (Coroneo et al., 2017). These reported concentrations, found in some market samples, were up to 14 and 34 times higher, respectively, than the limits set by the United States (i.e., 0.08 mg/kg) and Italy (0.03 mg/kg) for drinking water.

Additionally, it is known that citric acid is capable of reducing free chlorine concentrations in wash waters. Hence, processors are required to continually amend wash waters with additional NaOCl to maintain sanitizer efficacy. When this occurs, additional chlorine by-products are formed, which accumulate as a result of chlorine reacting with citric acid and organic materials (Fan & Sokorai, 2015). The accumulation of chlorine products ultimately leads to high absorption of chlorine by-products by fresh and fresh-cut produce (Gil, Marín, Andujar, & Allende, 2016; Nitsopoulos, Glaumer, & Friedle, 2014). Alternative pH control agents to citric acid are needed to minimize generating chemical by-products and stabilize free chlorine levels in wash waters.

While citric acid is most often used as a pH adjustor by the fresh produce industry, many other common organic acids are available, including malic, acetic, lactic, tartaric, adipic, ascorbic, formic, propionic, levulinic, succinic, and fumaric acid (Gurtler & Mai, 2014). Many of these organic acids are generally recognized as safe compounds (GRAS) by the U.S. Food and Drug Administration and have also been studied and applied by the food industry as acidulants, flavoring agents, preservatives, chelators, and antioxidants (Theron & Lues, 2007). Levulinic acid is a keto acid that has attracted attention in recent years due to its efficacy in pathogen inactivation on a number of foods (Zhou, Doyle, & Chen, 2020). It is unclear if these other organic acids will react with chlorine in the same manner as citric acid, forming trichloromethane or other chlorine by-products. In addition to organic acids, inorganic acids may also be used to adjust the pH of chlorinated water, although they may have a greater deteriorative effect on processing equipment. For example, the chlorine stabilizer SmartWash® (formerly known as T-128), used to stabilize and maintain chlorine levels in wash water, contains phosphoric acid (an inorganic mineral acid) (Nou et al., 2011), in conjunction with propylene glycol, used to stabilize chlorine concentrations (Shen et al., 2012). Earlier reports indicated that phosphoric acid may be used as an alternative to citric acid to reduce the pH in chlorinated wash water, while simultaneously limiting the amount of trichloromethane generated (Fan & Sokorai, 2015; Marín et al., 2020). Although it is predicted that other inorganic acids will not promote the generation of chlorine by-products, no published studies have confirmed this hypothesis. Previous studies on the reaction of chlorine with acids were often conducted at ambient temperatures, thus the extent of reactivity of acids

with chlorine at temperatures often used by the produce industry (e.g., 2–4°C) is unclear. The major objective of this study was to determine which acids generate low concentrations of trichloromethane, while still maintaining requisite chlorine levels. The two specific objectives of this study were to: (1) evaluate the stability of chlorine when combined with potential pH control agents at 3 and 22°C, and (2) to determine the ability of the pH control agents to generate chlorine by-products when reacting with chlorine at these two temperatures.

Materials and Methods

Sources of chemicals and standards. Trichloromethane (chloroform, high purity) was purchased from American Burdick and Jackson (Muskegon, MI). Citric acid (99%), L-tartaric acid (≥95%), acetic acid (≥99.7%), phosphoric acid (85%), levulinic acid (98%), DL-malic acid (99%), propionic acid (≥99.5%), sodium thiosulfate (99%), propylene glycerol (96%), 1-bromo-3-chloro propane (99%), and sodium phosphate (>99%) were purchased from Sigma-Aldrich (St. Louis, MO). Lactic acid (98%), ascorbic acid (99–100.5%), and sodium erythorbate (98.0–100.5%) were from Spectrum Chemicals (Gardena, CA). Adipic acid (99%) and succinic acid (99%) were purchased from Acros Organics (Pittsburgh, PA, USA). Fumaric acid (95%) was purchased from MP Biomedicals (Santa Ana, CA), and sodium acid sulfate (i.e., sodium bisulfate) was donated by the Jones-Hamilton Company (Walbridge, OH, USA). Water, purified with a Barnstead E-pure purification system (Dubuque, IA, USA), was used to prepare all solutions in the present study.

Reactivity of acids with chlorine. A stock solution of free chlorine (~1 g/L, 1,000 ppm, or 0.1%) was prepared from 8.25% sodium hypochlorite (Clorox, Oakland, CA, USA) in purified water. The concentration of free chlorine was confirmed using the colorimetric DPD (N, N-diethyl-p-phenylenediamine) method (Hach Co., Loveland, CO, USA). Solutions of 10 mM acids (citric acid, malic acid, tartaric acid, acetic acid, lactic acid, propionic acid, levulinic acid, ascorbic acid, sodium erythorbate, dehydroascorbic acid, adipic acid, fumaric acid, succinic acid, and sodium acid sulfate) were prepared in Na-phosphate buffer (10 mM). The pH of the solutions was adjusted to 6.5 using HCl or NaOH solutions (1 or 0.1 mM). The pH (6.5) was chosen to mimic commercial practices in which the pH of chlorine solutions is often maintained between pH levels of 6 and 7 for antimicrobial effectiveness and minimizing off-gassing, and resultant strong odors (Suslow, 1997). These acids were chosen because they are common acids, either as FDA-approved acidulants, currently used, or have FDA GRAS status. Chemicals with the highest purity available were used to minimize possible interference from impurities. Phosphate buffer was used to maintain the pH after the addition of chlorine. Water and Na-phosphate buffer (0.01 mol/L) were also tested as negative controls.

Temperatures of the stock chlorine and acid solutions were equilibrated at 22 and 3°C (i.e., 2–4°C) (on ice) before being mixed, to reach a targeted free chlorine concentration of ~100 mg/L by diluting 1 mL of the chlorine solution into a 9 mL acid solution. Concentrations of free chlorine were measured immediately (~1 min) and every hour thereafter, during a 3-h incubation period at both 3 and 22°C, using the DPD test method following 1:100 dilutions. The two temperatures were tested because most fresh produce items (especially fresh-cut produce) are often handled and washed in chlorinated water at temperatures of ≤4°C, while some fresh produce items are still washed at ambient room temperature.

Trichloromethane generation from chlorine reacting with organic acids. The previously described chlorine stock solutions (0.1 mL) were mixed with 0.9 mL of each respective acid solution in 7 mL glass vials (15 × 45 mm) to obtain free chlorine concentrations of ~100 mg/L in the mixtures. The vials were then sealed with caps

lined with PTFE septa (Thermo Fisher Scientific, Waltham, MA, USA). Following storage for 30 min at 3 and 22°C, 20 µL of a 2% sodium thiosulfate solution was injected into each vial, using a syringe, to neutralize the residual chlorine. The 30-min reaction time was chosen to accommodate for the gas chromatograph mass spectrometer (GC-MS) run time and subsequent analyses. An internal standard (10 µL) of a 15 mg/L concentration of 1-bromo-3-chloropropane, was injected into the vials through the septa prior to analyses. To test for concentrations of the chlorine by-products, vials were placed into the holes of a heating block held at 35°C. After 25 min, a carboxen/polydimethylsiloxane solid phase microextraction (SPME) fiber (85 µm coating, Stableflex, Sigma-Aldrich) was inserted into the headspace of the vials for 25 min to absorb volatile compounds. During incubation and absorption periods, liquid in the vials was mixed using a mini magnetic stir bar and placed on a magnetic stir plate at a speed of 700 rpm (model# SP133835, Barnstead Intern., Dubuque, IA, USA). The fiber was then injected into the GC injection port (at 250°C) with a desorption time of 4 min. Separation of compounds was achieved using DB-5MS (30 m, 0.32 µm ID, and 1 µm film thickness) capillary column coupled with an Agilent 6890 GC and 5973 MSD (Agilent Technology, Santa Clara, CA, USA). Helium was used as a carrier gas and set at a constant flow rate of 1 mL/min. The oven temperature was programmed to 40°C for 1 min, increased to 70°C at 5°C/min, then to 250°C at 25°C/min, and finally held at 250°C for 6 min. The MS source temperature was 230°C, and the mass spectra were recorded in full scan mode. The compounds were identified by comparing the retention times and mass spectra with the mass spectra of pure compounds with the assistance of the NIST version 02 library. Trichloromethane levels in the samples were quantified with standard curves.

Establishment of a trichloromethane standard curve. Stock solutions of trichloromethane (~10 µL) were injected into 1 mL of ethanol in 2 mL glass vials through the septum. The mass of the compound in the vials was weighed and recorded. Working standard solutions were further prepared by diluting the stock solutions in purified water. Next, a series of standard concentrations (0–500 µL) was prepared in 7 mL glass vials to establish calibration curves. Vials were sealed with PTFE-faced septa under open screw-top caps. Chlorine by-products in the solutions were measured using the SPME-GC-MSD method, as described above.

Statistical Analysis. All experiments were conducted in three independent biological replicates, while each experiment was conducted on separate days. Data were subjected to SAS analysis (version 9.2, SAS Institute, Cary, NC, USA) using the general linear model (GLM) procedure. The significance of treatment effect was determined by separating means with Duncan's Multiple Range Test at a significant level of $P < 0.01$.

Results and Discussion

Reactivity of acids with chlorine. Ascorbic acid and dehydroascorbic acid reacted readily with chlorine at 22°C, resulting in the rapid reduction of free chlorine levels (Fig. 1). Within minutes of mixing, more than half of the 100 mg/L of chlorine was consumed by ascorbic acid and dehydroascorbic acid. After 2 h at 22°C, free chlorine concentrations fell to levels close to or lower than the detection limit (i.e., 0.1 mg/L). Sodium erythorbate, an analog of ascorbic acid, commonly used by the food industry as an antioxidant in meat products (Barringer, Abu-Ali, & Chung, 2005), was even more reactive with chlorine. Chlorine was completely depleted within a few minutes. Therefore, unadvisable for these three compounds to be used to regulate the pH of chlorinated water, although ascorbic acid is a natural constituent of fresh produce, and is used to inhibit the browning of some fruits and vegetables (Fan, Gurtler, & Mattheis, 2023).

Citric acid and malic acid also reacted with chlorine with significant reductions in free chlorine levels during the first hour (Fig. 1).

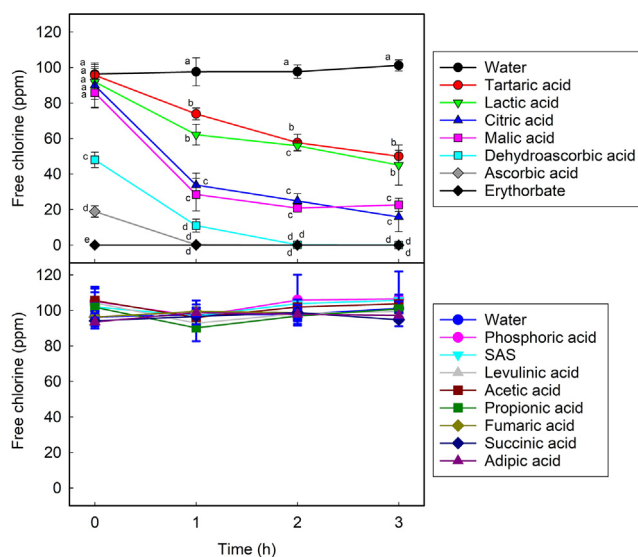


Figure 1. Free chlorine concentrations (reported in ppm, mg/L) in chlorinated water containing various pH regulators at pH 6.5 during a 3-hour storage at 22°C. Vertical bars represent standard deviations ($n = 3$). Symbols with the same letters at the same testing time are not significantly different (Duncan's multiple range test, $P > 0.01$).

There was no significant ($P > 0.01$) decrease after 1 h and no apparent difference between the two acids in reducing chlorine concentrations. Free chlorine concentrations, after mixing with citric acid and malic acid, were significantly higher than those mixed with ascorbic acid or dehydroascorbic acids at all four testing times (Fig. 1).

Chlorine was consumed by tartaric acid and lactic acid at rates of 15.3 and 14.7 mg/L per hour, respectively (Fig. 1). The decline in chlorine levels was linear during the 3 h incubation period at 22°C, with R^2 of 0.96 and 0.89 for tartaric acid and lactic acid, respectively. Following 3 h of storage with tartaric and lactic acids, chlorine levels were ca. 50% of their initial concentrations. Other pH regulators, including acetic acid, propionic acid, levulinic acid, fumaric acid, adipic acid, succinic acid, phosphoric acid, or sodium acid sulfate, did not significantly affect free chlorine concentrations during the 3 h. At the same molar equivalent concentration (10 mM) of acids, the reactivity of the pH control agents with 100 ppm free chlorine decreased in the following order: ascorbic acid = dehydroascorbic acid > citric acid = malic acid > lactic acid = tartaric acid > other acids and agents.

Similar to results at 22°C, ascorbic acid, sodium erythorbate, and dehydroascorbic acid reacted with chlorine rapidly at 3°C as well (Fig. 2). Free chlorine concentrations decreased linearly ($R^2 = 0.96, 0.97$) over time due to the presence of citric or malic acids at rates of 15.1 and 14.7 mg/L free chlorine per hour, respectively. Compared with the reductions in free chlorine concentrations at 22°C (i.e., 23.1 and 19.7 mg/L free chlorine per hour, respectively), free chlorine reductions as affected by the presence of citric acid and malic acid were significantly slower at 3°C. Other pH control agents, however, did not significantly reduce free chlorine levels during 3 h of incubation at 3°C (Fig. 2). Although tartaric acid and lactic acid were found to reduce free chlorine levels at 22°C, no significant effect on chlorine concentrations was observed at 3°C.

In our present study, the reaction of acids with chlorine was monitored for 3 h, and significant reductions in chlorine concentrations occurred at 3°C, in the presence of citric acid. Although the water used to wash free produce by the industry is not completely replenished, wash tanks are often recharged with fresh water at rates of 10–50% (Alharbi et al., 2017), while the majority of fresh produce wash water is recirculated and reused (Zhang, Luo, Zhou, Teng, & Huang, 2022).

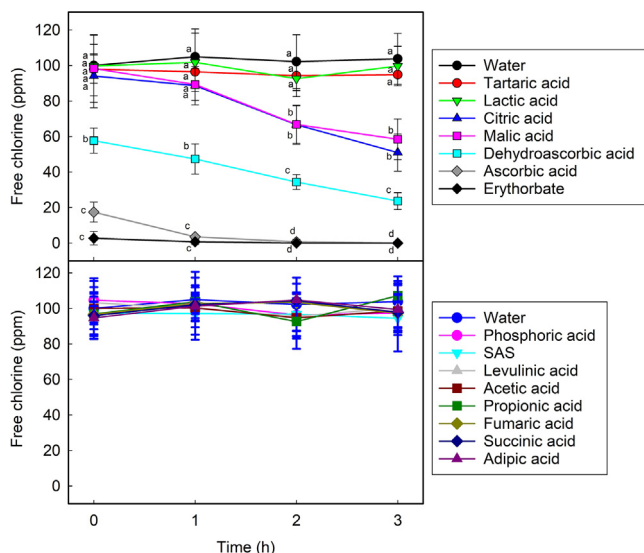


Figure 2. Free chlorine concentrations (reported in ppm, mg/L) in chlorinated water containing various pH regulators at pH 6.5 during a 3-hour storage at 3°C. Vertical bars represent standard deviations (*n* = 3). Symbols with the same letters at the same testing time are not significantly different (Duncan’s multiple range test, *P* > 0.01).

Longer dwell times, prior to replenishing water, may lead to more chlorine consumption as demonstrated in the present study (Fig. 2), subsequently leading to potentially higher accumulation of chlorine by-products (see below).

Generation of trichloromethane from acids reacting with chlorine. At 22°C, trichloromethane concentrations, resulting from citric and malic acid reacting with chlorine, were significantly higher than those of other acids, while citric acid resulted in much higher concentrations of trichloromethane than malic acid, at this temperature (Table 1). No significant concentrations of trichloromethane were produced from chlorine in the presence of other pH control agents. Interestingly, the addition of ascorbic acid and sodium erythorbate did not lead to the generation of trichloromethane, even though the concentrations of chlorine were reduced immediately after addition of the acids. Similarly, lactic acid and tartaric acid did not induce significant

Table 1
Concentrations of trichloromethane (i.e., chloroform) (ppb, µg/L) from 100 ppm of free chlorine reacting with acids and other acidifiers adjusted to pH 6.5 for 30 min at 22 and 3°C

Acid	22°C	3°C
Water	2.4 ± 0.6cz ^a	2.5 ± 2.6bz
Ascorbic acid	4.8 ± 1.4cz	12.7 ± 10.7bz
Erythorbate	1.2 ± 0.6cz	2.0 ± 1.1bz
Malic acid	186.4 ± 103.4bz	24.7 ± 8.7by
Citric acid	1938.2 ± 622.0az	167.9 ± 58.0ay
Lactic acid	4.8 ± 0.6cz	2.0 ± 1.1bz
Tartaric acid	2.8 ± 1.8cz	3.6 ± 4.2bz
Acetic acid	2.4 ± 0.4cz	1.6 ± 0.9bz
Levulinic acid	5.6 ± 0.8cz	8.8 ± 1.5bz
Propionic acid	2.8 ± 0.6cz	2.1 ± 1.4bz
Fumaric acid	2.9 ± 0.6cz	2.6 ± 0.8bz
Succinic acid	3.2 ± 0.7cz	2.5 ± 1.2bz
Phosphoric acid	2.0 ± 0.8cz	4.1 ± 5.6bz
Sodium acid sulfate	2.8 ± 1.8cz	1.5 ± 0.7bz

^a The numbers are means ± standard deviations (*n* = 3). Means with the same letters in the same column (a-c) or the same row (z-y) are not significantly different (*P* = 0.01).

generation of trichloromethane from chlorine, although the two acids significantly reduced free chlorine concentrations.

At 3°C, the highest concentrations of trichloromethane in chlorinated water were generated via citric acid, while other acids did not form significant concentrations of trichloromethane at this temperature, when compared with the control. It should be pointed out, however, that low concentrations of trichloromethane already are present in the commercial bleach (sodium hypochlorite) itself, as indicated by its concentration in the negative control water-only samples.

Three additional chlorine by-products were also tentatively identified in chlorinated water containing citric acid, ascorbic acid, and sodium erythorbate (data not shown). These by-products were all chloropropanones, including 1-chloro-2-propanone, 1,1-dichloro-2-propanone, and 1,1,1-trichloro-2-propanone. The three compounds are also known as chloroacetone, 1,1-dichloroacetone, and 1,1,1-trichloroacetone, respectively. However, no chloroacetones were found in chlorinated water when the pH was adjusted with the other tested pH control agents. Previous research has identified the presence of chloropropanones in the wash water of fresh produce (Lee & Huang, 2018; Zhang, Lee, Luo, & Huang, 2022).

Free chlorine concentrations in water were measured by the DPD colorimetric method. Although this method is commonly used, it tends to result in large variations among samples, especially following 1:100 dilutions. Nevertheless, clear trends, with statistically significant differences, were observed in both the chlorine quenching and by-product results.

Our results confirm previous reports that citric acid rapidly depletes chlorine and forms trichloromethane (Fan & Sokorai, 2015; Marín et al., 2020). It has been proposed that trichloromethane is formed through several intermediate compounds (Suh & Abdel-Rahman, 1985). The generation of trichloromethane and other disinfection by-products is influenced by many factors such as pH, temperature, contact time, organic matter, and concentration of free chlorine (Doederer, Gernjak, Weinberg, & Farré, 2014). The pH of water has a significant effect on the generation of chlorine by-products. Often, a reduction in trichloromethane concentrations generated is commensurate with lower pH of the wash solution (Hung, Waters, Yemmireddy, & Huang, 2017). In our present study, the pH was maintained at 6.5; however, at more acidic pH values, less trichloromethane would be formed. It has been reported that gallic acid, caffeic acid, and most amino acids had the greatest capacities for depleting chlorine levels, requiring concentrations in the range of only 10 µmol/L or less to deplete 5 mg/L free chlorine by 50% within 1.5 min at ambient temperature (Toivonen & Lu, 2013). Pyruvic, ascorbic, chlorogenic, malonic, and oxalic acids, however, had slightly lower chlorine quenching capacities. Our present results demonstrate that ascorbic acid, dehydroascorbic acid, and sodium erythorbate had the highest chlorine quenching capacity among the compounds that were tested. However, it should be noted that we used higher initial chlorine concentrations (viz., 100 mg/L) compared to the earlier report (5 mg/L) (Toivonen & Lu, 2013).

Although malic and citric acids have similar structures and both deplete chlorine rapidly, citric acid forms much higher concentrations of trichloromethane. Despite the present results, and a report indicating that chlorination of malic acid generates trichloroacetaldehyde at pH 7 (Chang, Streicher, Zimmer, & Munch, 1988), the complete ramifications regarding chlorine reacting with organic acids are still unknown. It is unclear what contributes to the variation in the reactivity with chlorine among the organic acids, perhaps related to their antioxidant properties. It is known that some organic acids, such as citric acid, are antioxidants, scavenging reactive oxygen species through the transfer of hydrogen atoms (Ryan et al., 2019). Antioxidants readily react with free chlorine, which is a strong oxidant. Further studies may investigate the mechanism of trichloromethane and other chlorine by-product generation due to free chlorine reacting with organic acids. Common organic acids in fruits and vegetables

include malic, citric, and tartaric acids, all of which have similar carbon lengths and structures. Among these three fruit acids, tartaric acid had the least chlorine-quenching ability. Other organic acids, including short-carbon organic acids, such as acetic and propionate, did not lead to significant reductions in free chlorine levels. Our results demonstrate that major organic acids (citric acid and malic acid) as well as ascorbic acid, a common ingredient of fruits and vegetables, react readily with chlorine. When chlorine is used to sanitize fresh-cut fruits and vegetables, citric acid, malic acid, and ascorbic acid leach from cut surfaces and react with free chlorine, in addition to chlorine reacting with the acids on the cut surfaces themselves. Therefore, it may be prudent to use other sanitizers, such as peracetic acid, to treat some fresh-cut fruits, such as oranges and kiwi fruit, which contain high concentrations of ascorbic acid and other organic acids, such as citric acid, in order to minimize the formation of chlorine by-products.

Our results, in this study, demonstrate that citric and malic acids react with chlorine, forming high concentrations of trichloromethane, while other pH control agents, including phosphoric acid and sodium acid sulfate, did not react with chlorine or form trichloromethane, suggesting that those compounds may be used as pH control agents, as alternatives to citric acid. However, whether these compounds will be effective pH control agents largely depends on their individual buffering capability and pKas. The buffering capacity of an acid affects how well pH will be maintained, and acid has the greatest buffering capacity when the pH of the acid solution is the same as that of its pKa. The pKa values of most pH control agents used in the present study are below 6 (except for phosphoric acid and citric acid) (Table 2), indicating that they are not good pH control agents for maintaining a pH of 6–7 in chlorinated water. Based on the pKas of the pH control agents tested in this study, phosphoric acid would be the best pH control agent for buffering the pH of 6–7 of chlorinated water. In recent years, chlorinated water with a pH of 5.5 has been used by some fresh produce processors, probably to maximize the active form of chlorine (hypochlorous acid), without inducing off-gassing (the generation of chlorine gas) (Marín et al., 2020; Zhang, Lee, et al., 2022). If a pH of 5.5 is desirable, other organic acids, such as adipic acid or succinic acid, with pKas of 5.4 and 5.6, respectively, may be used in place of citric acid. Our results also revealed that sodium acid sulfate (sodium bisulfate) did not react with chlorine or induce the formation of chlorine by-products. However, sodium bisulfate has a pKa of 2.0, suggesting it has little buffering capacity in the pH range of 5–7.

Future studies may be conducted to determine if these two acids, used as pH control agents in chlorinated water, would perform better than citric acid to prevent pathogen cross-contamination in the presence of fresh produce organic matter. The presence of organic matter,

leaching from fresh produce, will affect the formation of disinfection by-products. As shown in our previous study (Fan & Sokorai, 2015), in the absence of organic matter, citric acid reacted with chlorine, leading to the formation of trichloromethane. However, in the presence of organic matter, organic compounds competed with citric acid and reduced free chlorine concentrations, resulting in less production of trichloromethane. More studies are needed to evaluate the formation of disinfection by-products as a result of the reaction of pH control agents with chlorine in the presence of organic matter. In addition, potential effects on the sensory quality of washed fresh produce may be investigated.

Further studies are also needed to determine if other pH regulators can form additional disinfectant by-products, particularly in the presence of organic matter from fresh produce, using methods more sensitive than the SPME method used in the current study. This conclusion is based on the fact that there are many other species of chlorine by-products that can be generated in chlorinated water in fresh-cut processing plants, such as haloacetic acids, nitrogenous, and other carbonaceous disinfection by-products (Zhang, Lee, et al., 2022). For example, an earlier study (Marin et al., 2020) reported that citric acid in chlorinated water accumulated lower concentrations of haloacetic acids, even though trihalomethane levels were higher in the presence of citric acid, in comparison with other inorganic pH-reducing agents (pH 5.5) (Marín et al., 2020).

In summary, our results demonstrate that citric acid and malic acid consume chlorine rapidly, with significantly higher rates at 22°C than at 3°C. Lactic acid and tartaric acid significantly reduced levels of free chlorine at 22°C, but not at 3°C during the 3 h period. All other pH control agents did not significantly react with chlorine at either temperature. Among the acids that rapidly depleted chlorine, only citric acid generated high amounts of trichloromethane. Furthermore, chloroacetones were formed from chlorine in the presence of citric acid, ascorbic acid, and sodium erythorbate. Using phosphoric acid or sodium acid sulfate as pH control agents did not result in the depletion of chlorine or the generation of trichloromethane or chloroacetones. Our results demonstrate that citric acid can be replaced with inorganic acids and other organic acids with pKa values of 5–7, to regulate the pH of chlorinated fresh produce wash waters, while preventing the formation of trihalomethane.

CRedit authorship contribution statement

Xuetong Fan: Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Joshua B. Gurtler:** Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2
Dissociation constants (pKa) of pH control agents in aqueous solutions

pH control agents	pKa	pH control agents	pKa
Ascorbic acid	4.7 ^a	Acetic acid	4.8 ^e
Dehydroascorbic acid	3.9 ^b	Propionic acid	4.9 ^e
Levulinic acid	4.6 ^c	Fumaric acid	3.0, 4.4 ^e
Sodium erythorbate	4.0, 11.3 ^d	Adipic acid	4.4, 5.4 ^e
Citric acid	3.1, 4.8, 6.4 ^{de}	Succinic acid	4.2, 5.6 ^e
Malic acid	3.4, 5.1 ^e	Phosphoric acid	2.2, 7.2, 12.3 ^f
Tartaric acid	3.0, 4.3 ^e	Sodium acid sulfate	2.0 ^g
Lactic acid	3.1 ^e		

^a National Library of Medicine. (2024a).

^b National Library of Medicine. (2024b).

^c National Library of Medicine. (2024c).

^d EFSA Panel on Food Additives and Nutrient Sources added to food. (2016).

^e Doores, S. (2005).

^f Falcon-Millan et al., (2017).

^g Lachenwitzer et al., (2002).

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