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Storage-Mediated Changes in Sodium Hypochlorite and Peroxyacetic Acid Sanitizer Concentrations

ABSTRACT

Chlorine and peroxyacetic acid (PAA) are widely used throughout the food industry as economical and effective sanitizers. However, efficacy is partially dependent on proper storage conditions, as they are affected by UV light exposure, temperature, and pH, but the degree to which these factors influence sanitizer effectiveness has not been well described. This study examined the effects of plastic bottle type (transparent or opaque) and storage conditions on chlorine (200 ppm of free chlorine) and PAA (60 to 80 ppm) concentrations. Bottles were stored in outdoor, indoor, or refrigerated conditions, and sanitizer concentrations were measured over a 32-day period (summer 2022). Although bottle type significantly affected chlorine stability, it did not result in a significant reduction in PAA concentration. The free chlorine concentration in sanitizers stored in translucent bottles was depleted by day 4 of outdoor storage and ca. 50 ppm by day 13 when stored at room temperature. PAA concentrations stored outdoors or at room temperature ranged from ca. 0 to 30 ppm by day 32. Both sanitizer concentrations remained consistent under refrigeration for all bottle types through the 32-day period. Proper storage and monitoring of sanitizer can ensure adequate microbial reduction when used in food handling environments.

INTRODUCTION

Adequate cleaning and sanitizing procedures are critical to reduce food safety risks in produce growing and packing environments. These two distinct procedures are necessary to remove soil (cleaning) and reduce or eliminate microbial hazards (sanitizing) on food-contact surfaces, respectively *(16, 19, 21, 26, 38)*. Sanitizing of a food-contact surface may only occur after the area has been thoroughly cleaned, and chemicals used must be labeled for use on food-contact surfaces (21 Code of Federal Regulations section 178.1010). In the United States, sanitizers are regulated as pesticides by the Environmental Protection Agency and bear a label describing instructions for preparation and use *(29)*.

Instruction on cleaning and sanitizing is complicated by a variety of factors in the produce industry, particularly high employee turnover *(25)*. In a survey of growers who washed

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the produce after harvest, 34% (15 of 44) either used an incorrect concentration of sanitizer in the wash water or did not know the concentration they used *(7)*. A survey of small- and medium-sized farms and farmers markets in Georgia, South Carolina, and Virginia found that only 39% (88 of 226) sanitize produce food-contact surfaces at the farm and only 33% (75 of 226) reported sanitizing produce transport containers. When asked for the sanitizers used, respondents reported using a variety of chemicals and concentrations unsuitable for sanitizing food surfaces *(11)*.

Two commonly used sanitizers in the food industry are sodium hypochlorite (bleach) and peroxyacetic or peracetic acid (PAA). Free or available chlorine, in the form of hypochlorous acid (HOCl) and hypochlorite ions (OCl^-), is the active compound in sodium hypochlorite-based sanitizers *(4)*. The availability of these compounds depends on pH, temperature, and UV radiation *(8, 24)*, which can affect the rate of ion dissociation. HOCl is the more active sanitizing agent, and a lower pH that favors HOCl in the solution instead of OCl^- will be a more effective sanitizer, though too low a pH will result in the formation of toxic chlorine by-products *(10, 20)*. The sanitizer PAA is produced from a reaction between acetic acid and hydrogen peroxide *(15, 32)*. Hydrogen peroxide and PAA within the solution have a synergetic bactericidal effect, as well *(1, 3, 18)*. Like free chlorine species, PAA is also an oxidizer causing DNA, lipid, and cell membrane damage *(32)*. The stability of PAA in aqueous solutions is affected by temperature, where high temperatures decompose PAA at a faster rate *(17)*. Both sanitizers are effective at reducing pathogens on food-contact surfaces *(2, 12, 14)*. However, there are only a handful of studies examining proper sanitizer storage to maximize storage life and efficacy *(6, 9, 17, 33)*, often focusing on non food-contact applications. Although it has been suggested that sanitizer solutions in spray bottles should be remade every 24 h *(5)*, fewer data supporting this time frame or supporting potential shelf-life extension through storage management are available. This work seeks to provide data regarding best practices for sanitizer storage, including how recommendations for how frequently sanitizers should be remade under different storage conditions. Sanitizer concentration, temperature, pH, solar irradiance (for bottles stored outside) and oxidation-reduction potential (ORP) were measured for each sanitizer-treatment combination over 32 days.

MATERIALS AND METHODS

Sanitizer preparation and storage conditions

Two types of high-density polyethylene plastic spray bottles were used: translucent (28 oz, Mainstays) and opaque (16 oz, Cindy's Tool). Commercial bleach (7.5%; Pure Bright, KK International LLC, Ontario, Canada) and PAA (15%; Shield-Brite, Pace International, Wapato, WA) were used to prepare 200 ppm (free chlorine) and 60 to 80 ppm solutions, respectively. These concentrations are appropriate for food-contact surfaces, as defined in 21 Code of Federal Regulations section 178.1010, and are consistent with Environmental Protection Agency labeling for these products.

Fresh solutions of each sanitizer (600 ml) were prepared with deionized water for each bottle. The pH was not adjusted. Bottles were placed in respective experimental conditions either outside in full sun, indoors on a windowsill (20 ± 2°C), or in a glass door refrigerator (4 ± 1°C). These conditions were chosen to mimic possible storage locations at a farm or packing facility. Sanitizer levels were measured immediately after mixing, and at the same time of day on each sampling day, measurements were recorded.

Sanitizer concentration, ORP, pH, solar irradiance, and temperature

Free chlorine levels for chlorine-based sanitizers were measured by using an HI96771 chlorine meter (Hanna Instruments, Woonsocket, RI), FAS-DPD chlorine and bromine test kits (LaMotte Company, Chestertown, MD), and chlorine test strips (Bar Maid, Pompano Beach, FL, or Franklin Machine Products, Lumberton, NJ). The PAA concentration was measured by using a PAA titration kit (AquaPhoenix Scientific, Hanover, PA). The ORP was determined by using a metallic ORP-indicating electrode and Accumet XL600 benchtop meter (Thermo Fisher Scientific, Waltham, MA), and pH and temperature were measured by using an Accumet AB250 benchtop meter (Thermo Fisher Scientific). Average, maximum, and minimum outdoor air temperatures were accessed via the National Oceanic and Atmospheric Administration's Climate Data Online database (www.ncei.noaa.gov/cdo-web/). Solar irradiance data were retrieved from the National Renewable Energy Laboratory's National Solar Radiance Database for the dates of the study by using the grid (2 by 2 km) containing the address of the research building *(23, 27)*. Sanitizer level (meter for chlorine and titration for PAA) was compared against the sum of the global horizontal irradiance (GHI) during the sanitizer's storage time *(Table 1)*. The study was conducted across 32 days with measurements recorded at days 0, 1, 4, 6, 13, 20, and 32.

Statistical design and analysis

Four replications of this experiment were completed between July and September 2022. A single replication included 12 treatment bottle–sanitizer combinations (*n* = 12), with two controls (deionized water stored at room temperature in opaque and translucent bottles). Raw data were entered into Microsoft Excel (version 16.61.1, Microsoft Corporation, Redmond, WA) and then exported into R Studio (version 1.3.959) by using the *readxl* package. Data were analyzed and visualized with several R packages *(13, 34,*

TABLE 1. The average and cumulative GHI across all trials for each sampling day for sanitizer bottles in outdoor storage; cumulative average is the sum of the average GHI up to and including that day

Day	Average GHI	Cumulative average GHI
0[a]	182.93	182.93
1	271.96	454.89
2	286.56	741.45
3	298.96	1,040.41
4[a]	298.32	1,338.73
5	263.14	1,601.86
6[a]	249.49	1,851.35
7	233.43	2,084.78
8	216.48	2,301.26
9	253.74	2,555.00
10	210.43	2,765.43
11	196.96	2,962.39
12	233.50	3195.89
13[a]	266.33	3462.22
14	242.26	3,704.48
15	213.72	3918.20
16	155.61	4,073.81
17	225.05	4,298.86
18	257.98	4,556.84
19	254.84	4,811.69
20[a]	250.81	5,062.50
21	247.39	5,309.89
22	265.39	5,575.27
23	251.25	5,826.52
24	217.20	6,043.72
25	253.00	6,296.72
26	253.86	6,550.58
27	227.94	6,778.52
28	191.99	6,970.51
29	248.26	7,218.77
30	270.16	7,488.93
31	197.02	7,685.95
32[a]	261.43	7,947.37

[a]Days on which bottles were measured.

36, 37). Figures were created with the *ggplot2* package *(33)*. All comparisons among factors (e.g., bottle type, storage condition) were done by using a two-way analysis of variance followed by Tukey honest significance test ($P < 0.05$). Correlation was determined by using Pearson correlation coefficient.

RESULTS AND DISCUSSION

Average outdoor temperature across the course of the experiment was 24.8°C (range: 18.3 to 28.3°C). Average sanitizer temperatures at the time of sampling were 24.0°C (range: 16.5 to 35.1°C), 20.9°C (range: 15.7 to 24.2°C),

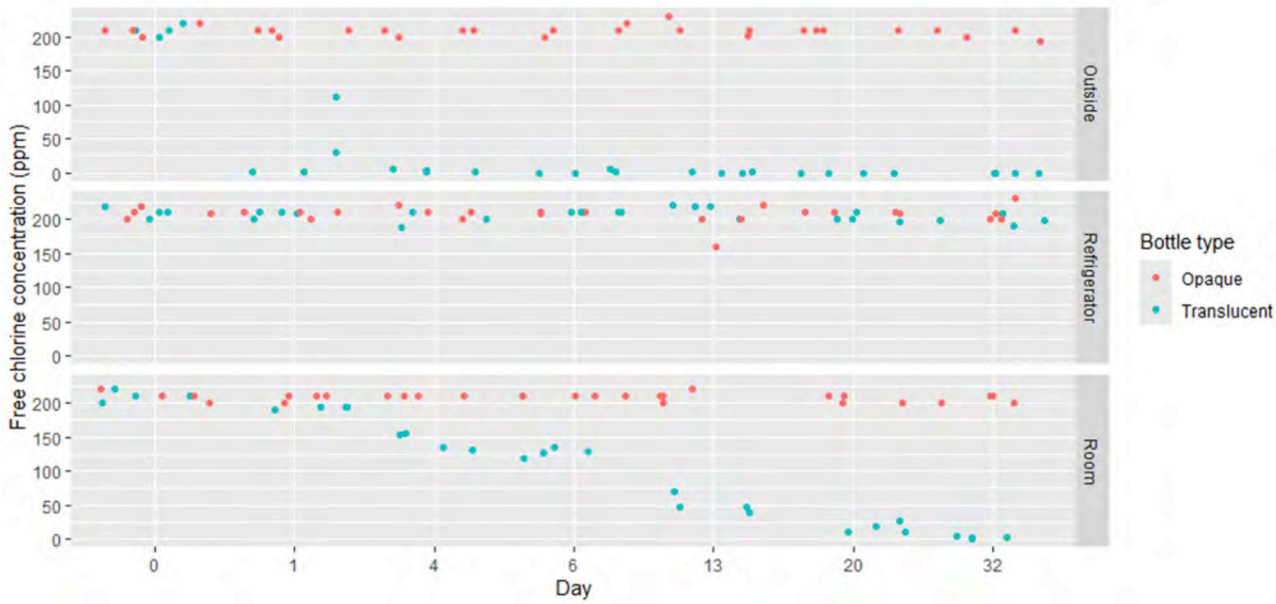

Figure 1. Change in chlorine concentration measured with a colorimeter over time by bottle type and storage condition. Two bottle types (translucent and black opaque) were stored outside under refrigeration (4°C) or at room temperature (20°C).

and 13.0°C (range: 7.0 to 22.2°C) for all sanitizer types stored outdoors, indoors, and in a refrigerator, respectively. There was a significant difference among average sanitizer solution temperatures across storage conditions ($F_{3,374} = 247$, $P < 0.001$). However, no significant differences in sanitizer temperatures by bottle type and sanitizer type ($F_{2,364} = 0.10$, $P > 0.05$) were found within the same storage conditions. Although storage conditions led to a significant difference among sanitizer temperatures, bottle type alone did not significantly affect sanitizer temperature.

Chlorine

The method (meter, titration, or strip) used to measure free chlorine concentration did not have a significant effect on the mean free chlorine concentration across all treatments ($F_{2,501} = 0.565$, $P > 0.05$). Although the free chlorine measurement method alone did not have a significant effect on mean free chlorine concentration, there was large variation in standard error of the mean free chlorine concentration between measurement methods grouped by bottle type and storage condition. For instance, chlorine sanitizers stored in translucent bottles and held at room or outdoor conditions had relatively large standard errors compared with that of opaque bottles stored at any condition or translucent bottles stored at refrigeration. This variability in mean free chlorine concentration may be caused by bottle type or storage condition and not measurement type.

Bottle type had a significant effect ($F_{1,502} = 238$, $P < 0.001$) on mean free chlorine concentration, with mean concentration of free chlorine in opaque and translucent bottles of 208 and 116 ppm, respectively. Storage condition also had a significant effect ($F_{2,502} = 55.4$, $P < 0.001$) on free chlorine concentration, where the mean free chlorine concentrations in bottles stored at refrigerator, indoor, and outdoor conditions were 206, 157, and 122 ppm, respectively. In addition, there was a significant interaction effect between bottle type and storage condition on the mean free chlorine concentration ($F_{2,486} = 145$, $P < 0.001$) *(Fig. 1)*. Chlorine levels for outdoor samples were significantly ($P <$ 0.05) affected by the cumulative GHI to that sampling date, $F_{(1,206)} = 7.63$ and differed significantly between the bottle types, $F_{(1,206)} = 37.98$. There was a significant interaction between bottle type and the effect of cumulative GHI, $F_{(1,206)} = 8.24$. Translucent bottles saw greater decreases in the level of chlorine than opaque.

In this experiment, a strong *(27)* Pearson correlation, 0.714, and 95% confidence interval (0.630, 0.781) were observed between ORP and free chlorine measured by meter. However, some inconsistency was also observed. For instance, although chlorine sanitizer concentration decreased steadily over time in translucent bottles stored indoors, the ORP remained between 550 and 700 mV *(Fig. 2)*. Although ORP can provide an indication of the availability of oxidizing agents within a sanitizer, with higher ORP typically indicating greater oxidation potential of the sanitizer, other work has shown it to provide an unreliable estimate of sanitizer concentration *(29)*. Therefore, despite a strong correlation overall, reliance on ORP measurements may not provide an accurate indication of sanitizer concentration across all conditions.

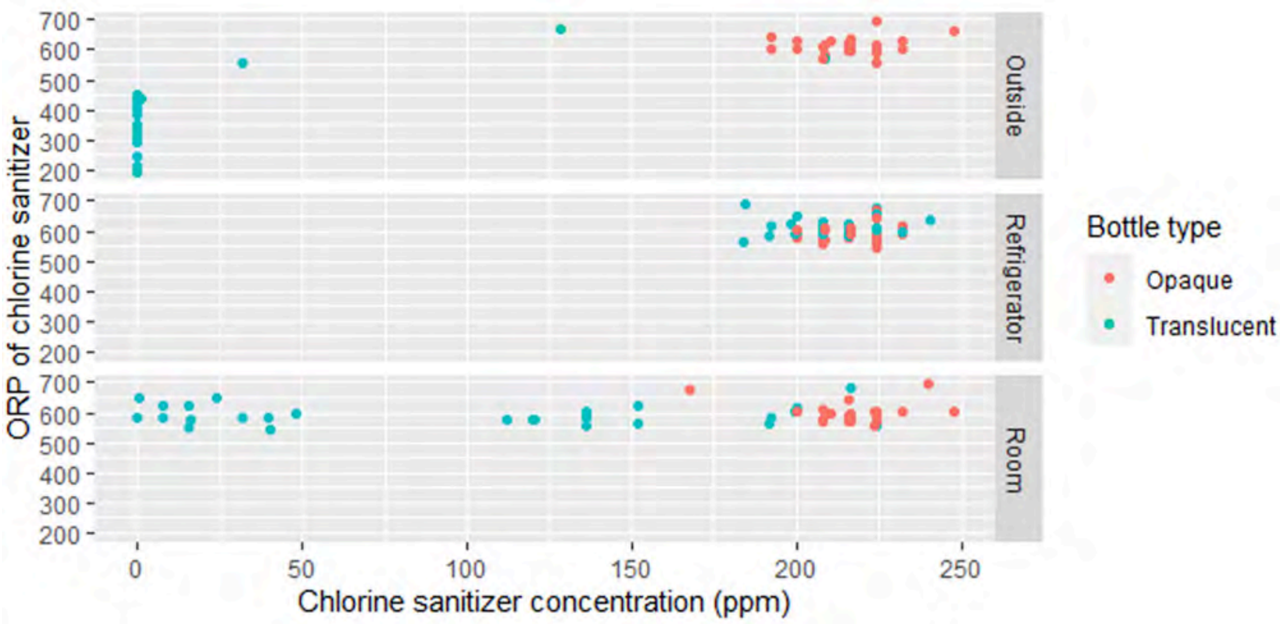

Figure 2. Change in chlorine sanitizer ORP by bottle type and storage condition. Two bottle types (translucent and black opaque) were stored outside under refrigeration (4°C) or at room temperature (20°C).

The formulation pH can have a profound impact on the antimicrobial efficacy of chlorine-based sanitizers, as solutions with pH below 6 or above 8 can lose efficacy *(20)*. When sodium hypochlorite and other chlorine compounds are added to water, they dissociate into HOCl, the primary antimicrobial species due to its high oxidative activity, and −OCl, a less effective antimicrobial compound. When the pH of the solution is around 7 or lower, a greater proportion of HOCl is formed, but as the pH increases above 7, −OCl becomes the predominant species, greatly diminishing antimicrobial efficacy of the overall solution. Throughout the study, free chlorine measurements, which indicate the combined HOCl and −OCl in a solution, indicated the solutions were adequate for use on food-contact surfaces between days 0 and 1 for all storage conditions and bottle types. However, when mixed to a concentration of 200 ppm of free chlorine according to Environmental Protection Agency label instructions, the pH of the chlorine solutions was 9.8. This high initial pH indicates that users do need to adjust pH to around 6.5 to 7.5 when chlorine solutions are mixed for food-contact surface sanitation *(10, 20)*. This can be done by adding a food grade acid, such as acetic or citric acid, to the mixed chlorine solution until the pH is within the ideal range to ensure most of the free chlorine is present as HOCl.

Unfortunately, more complex procedures in sanitation programs may be a barrier to employee adoption, especially in fast-paced environments *(25)*. Sanitizers that require multiple steps during mixing or dilution, especially for sanitizers that must be remade frequently, provide more opportunities for mistakes or intentional, improper procedures. The addition of a pH adjustment may be considered cumbersome and excluded when time constraints exist. Fortunately, all free chlorine measurement methods used in this study performed similarly, so less time-intensive monitoring methods such as test strips, which may be more economical and require less training, may improve adherence *(22)*. However, as with any measurement method used, test strips must be stored correctly and used according to manufacturer recommendations. Color blind or deficient users may be unable to discern the subtle color differences on many test strips and may need to rely on other measurement methods.

PAA

Change in PAA concentration was affected more by storage condition than bottle type *(Fig. 3)*. Although bottle type had a significant effect on chlorine concentration, it did not have a significant effect on PAA concentration ($F_{1,166} = 3.18$, $P > 0.05$). Storage condition, on the other hand, had a highly significant effect on PAA concentration ($F_{2,165} = 33.24$, $P < 0.001$). PAA sanitizer in bottles stored outdoors experienced the greatest decrease in PAA concentration, followed by those stored at room temperature, and finally by those stored in a refrigerator. There was a significant effect from the cumulative GHI, $F_{(1, 188)} = 35.9$, for bottles stored outdoors, but no significant effect from the type of bottle used, nor the interaction between bottle and cumulative GHI. Overall, bottle type

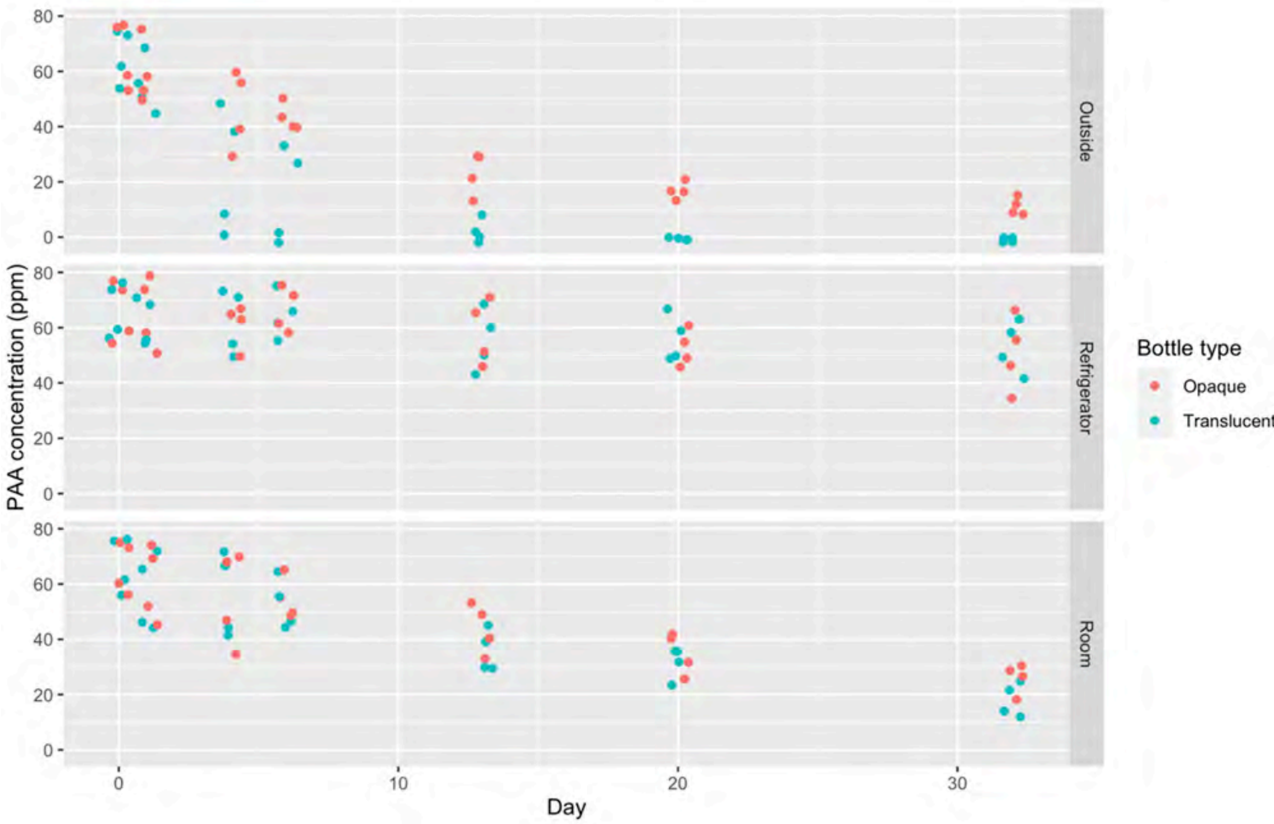

Figure 3. Change in PAA concentration over time by bottle type and storage condition. Two bottle types (translucent and black opaque) were stored outside under refrigeration (4°C) or at room temperature (20°C).

appears to be less important for extending the shelf life of PAA solutions than chlorine.

Multivariate analysis of variance results for a three-way interaction between condition, bottle type, and day indicated that all single effect and interaction effects were significant ($P < 0.01$), except for bottle type versus day and condition versus bottle type versus day. Although PAA sanitizers stored outdoors in translucent bottles decreased in concentration more rapidly compared with those in other storage conditions *(Fig. 3)*, this appears to be likely due to outdoor temperature rather than by bottle type. To maintain PAA sanitizer concentration for a longer period, it is recommended that bottles not currently in use be stored under refrigeration for no longer than 10 to 12 days to maintain effective concentrations for use on food-contact surfaces, around 40 to 80 ppm.

ORP measurements had a moderate *(27)* Pearson coefficient of 0.449 and 95% confidence interval (0.319, 0.563). However, there were individual measurements that deviated from the general range of 425 to 475 mV *(Fig. 4)*. Fluctuations in ORP readings around 25 mV are common in other food sanitation applications *(30)*. Although a continuous monitoring system relying on ORP measurements may not be significantly affected by these fluctuations, individual data points for monitoring (i.e., a single reading measured at mixing prior to filling a spray bottle) may be susceptible to incorrect estimations of sanitizer effectiveness. The variability in ORP when measuring both chlorine and PAA sanitizers suggests that alternative monitoring methods should be considered to ensure target levels are maintained.

Storage and other factors on the farm

Produce packinghouses range from being entirely nonclimate controlled, partially climate controlled, or entirely climate controlled, depending on the commodities packed, activities conducted at the packinghouse, packinghouse location, farm size, and many other factors. Therefore, storage conditions also vary among farms, with sanitizer storage ranging from outside with highly variable fluctuations to inside storage in cool temperatures with little variability. The conditions selected were intended to represent the conditions at farms throughout the southern United States: bottles stored in direct sunlight for a worst-case scenario; indoor storage by a window with some temperature and light variability, similar to the moderate

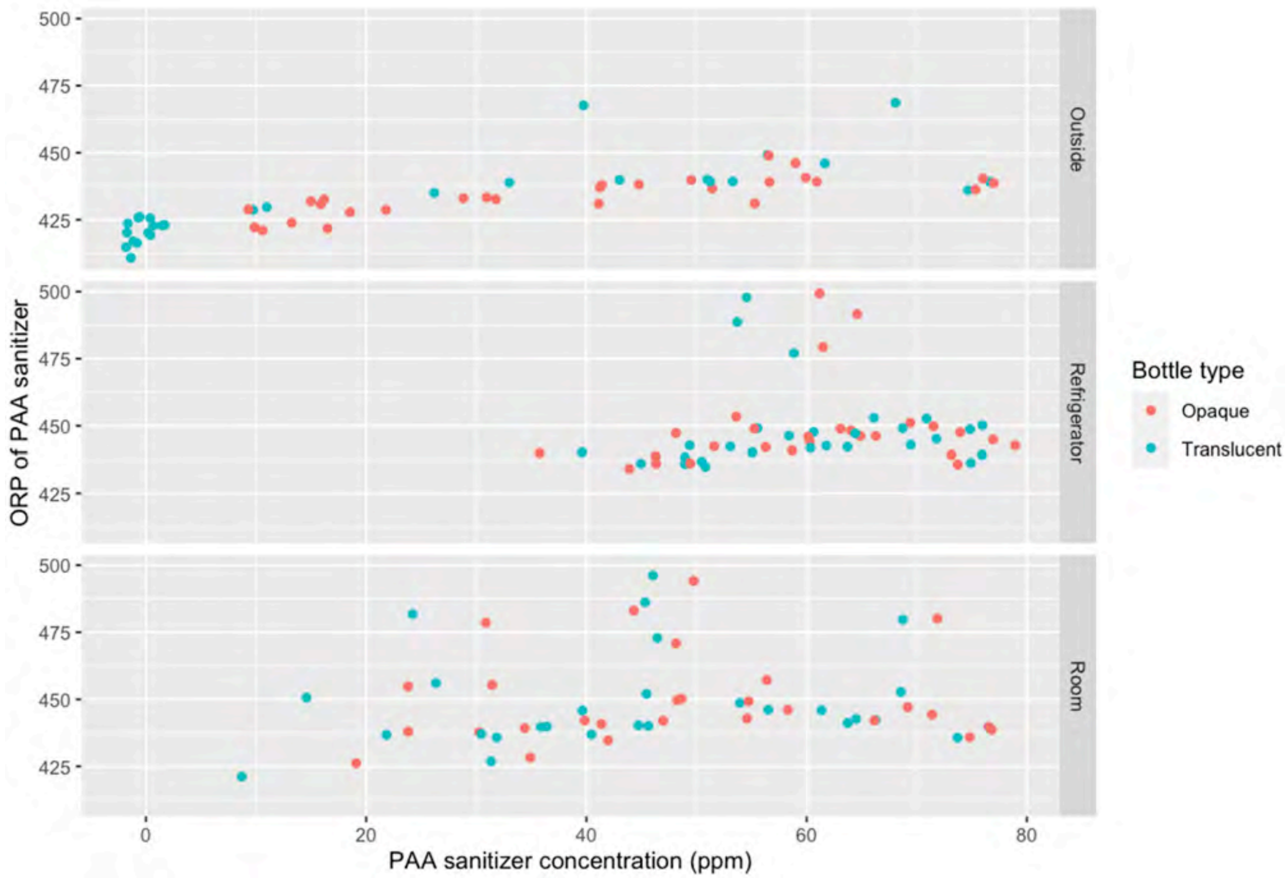

Figure 4. Change in PAA sanitizer ORP by PAA concentration, bottle type, and storage condition. Two bottle types (translucent and black opaque) were stored outside under refrigeration (4°C) or at room temperature (20°C).

variability in conditions that may occur in a climate-controlled or partially climate-controlled packinghouse; and under refrigeration, out of direct sunlight, illustrating the best-case storage scenario. Actual storage conditions for packinghouses may fall anywhere along the spectrum examined.

Deionized water was used in the study to reduce variability due to water sources. However, mineral content of a water source is known by the impact pH and chemical demand, which is particularly critical for free chlorine efficacy. Hard water, characterized by having a high mineral content, is typically also more alkaline than soft water, so packinghouses with hard water may require increased acidification of sanitizer solutions to increase HOCl in chlorine sanitizers. Minerals in water also bind chlorine added to the solution, resulting in an increased chlorine demand and reduced free chlorine *(31)*. A study examining the impact of water hardness on chlorine-based sanitizers against *Escherichia coli* O157:H7 determined that for short (10-s) exposure times to 0.5 ppm of sodium hypochlorite, the solution made with deionized water achieved an approximately 4.8 log CFU/ml reduction, while water with 1,000 and 5,000 mg/liter CaCO3 was 1.6 and 0.0 log CFU/ml, respectively. However, longer treatment did improve sanitizer efficacy at across most hardness levels *(31)*. Growers do not necessarily need to monitor mineral content of water sources, but operations spread across multiple growing regions should consider that different locations may require different volumes of sanitizer concentrate to achieve the same quantity of active compound in the final, mixed solution. This is yet another reason that final sanitizer solutions should be measured to ensure that adequate concentrations are achieved, instead of simply relying on the addition of a set sanitizer volume. Concentrations of both sanitizers examined in this study decreased over time. Though decrease could be mitigated with different storage conditions for the chlorine sanitizer *(Fig. 1)*, all storage conditions for PAA saw a decrease over time *(Fig. 3)*. Although preparing a new solution every 24 h *(5)* may ensure an appropriate level of sanitizer in most cases, the outside translucent bleach bottle may drop below an effective level well before. Replacement intervals should consider both the chemistry and the storage conditions of a chosen sanitizer instead of a blanket time.

CONCLUSIONS

Storage conditions, containers used, and solution age can affect the efficacy of sanitizer working solutions, which should be remade on a regular schedule or when monitoring indicates the solutions are no longer at an appropriate concentration. Monitoring both the free chlorine concentration and pH is essential when using hypochlorite sanitizers, and user-friendly measurement tools such as chlorine test strips appear to be comparable to more time-intensive and expensive methods (i.e., meters, titrations) when stored and handled properly. The protective effect of the opaque bottles suggests they could be an economical step taken to preserve the efficacy of bleach sanitizers, particularly when used in outdoor settings, but may be less critical when using PAA sanitizers.

Although chlorine is widely used and is generally less expensive, PAA may provide more flexibility, as it is not affected by bottle type or storage temperature, nor is a pH adjustment or measurement necessary. These factors have likely contributed to its increased adoption of PAA in the produce industry *(25)*. Because of the significant decline in concentration observed in outside storage, it may be prudent to recommend that PAA bottles be stored inside when possible or replaced after extended exposure to warm temperatures. Although ORP is frequently used to monitor sanitizer levels in some wash waters *(7)*, this study found less utility for ORP as a monitoring tool for sanitizers stored in spray or similar style bottles.

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