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Peracetic Acid and Hydrogen Peroxide Post-dip Decay Kinetics on Red Meat and Poultry

ABSTRACT

Antimicrobial processing aids are used to mitigate the food safety risk from pathogens that may be present on poultry and beef products. Although efficacy is well documented, post-application decay of the antimicrobial peracetic acid (and the equilibrium by-product hydrogen peroxide) has not been documented. This was the purpose of the study. Chicken and beef samples were dipped in solutions of > 2000 parts per million (ppm) peracetic acid for 30 seconds and then set on a drying rack for set time periods. After times ranging from 0.5 to 30 minutes, samples were assayed for residual peracetic acid and hydrogen peroxide content. The assay consisted of immersing the sample in water and shaking for 30 seconds to recover the residue. The rinse water was then titrated to measure residuals. Exponential decay kinetics were used to predict the time at which < LOD was reached for peracetic acid and peroxide on both chicken and beef samples. Because ln(0) is undefined, < LOD was defined as 0.1 to fit the model. Based on the fitted models, peracetic acid levels reached < LOD at 27.9 minutes for chicken, with a 95% confidence interval of (26.2,30.0), and 3.5 minutes for beef, with a 95% confidence interval of (3.1,4,0). Hydrogen peroxide levels reached < LOD at 29.1 minutes for chicken, with a 95% confidence interval of (27.4,31.0), and 12.3 minutes for beef, with a 95% confidence interval of (10.7,14.5). These results support the use of up to 2000 ppm peracetic acid antimicrobial interventions as processing aids in meat and poultry operations, with no long-term residues.

INTRODUCTION

Current methods for processing of freshly slaughtered poultry or beef rely on use of water in the process for various functions, including cleaning of the inside and outside of the carcass as well as chilling of the carcass to preserve meat quality and retard microbial growth. Recent data for water consumption rates limited to processing operations (not including facility cleaning) is not widely available; however, studies for broiler processors have reported a use range of 21–30 l/bird (all sizes) *(7)*, and a more recent study for beef processors conducted at a

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beef processing facility measured 470 l/1000 kg Live Body Weight for intervention processes *(14)*, which is a refinement and reduction from prior reports of 570–1700 l water/animal *(2)*. Although the mechanical action of water can provide some removal of surface microbial contamination that may naturally be present on the carcass, the use of water alone introduces a food safety risk, as a single carcass may cross-contaminate pathogenic bacteria to uncontaminated carcasses being processed at the same time or later *(6)*.

This use of water relates directly to the importance of critical control points in use at processing facilities for organisms of public health significance. To control both pathogenic and spoilage microorganisms naturally present on the meat surface and decrease the presence of microorganisms due to cross-contamination, the use of an antimicrobial agent(s) in process water is common. This method can achieve a reduction in microbial levels by several orders of magnitude. Options available for use in processing include oxidative chemicals, such as hypochlorite, acidified sodium chlorite, bromine, or peroxyacids; organic acids, such as lactic acid; and the quaternary ammonium antiseptic cetylpyridinium chloride *(11)*. Information on the use of each type of antimicrobial is lacking; however, the use of oxidative chlorinated interventions has declined for various reasons. Restrictions on meat or poultry processed with hypochlorite have been in effect since 2010 for Russia, and a ban on the use of pathogen reduction treatments other than water or lactic acid has been in effect for the EU since 1997 *(8)*. Import/Export restrictions are continually revised and subject to change, with the most recent list of approved or disapproved agents permitted for United States exported meat and poultry product use listed in the U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS) Export Library *(13)*.

Of the aforementioned options for microbial intervention, peracetic acid has almost certainly increased in usage over the past decade. Adoption and acceptance of its use is attributable to several factors, including broad allowance by beef and poultry importing countries, a large operating range with efficacy at low levels, a high tolerance for organic load, and the unique property, by comparison with the others, of a breakdown path resulting in innocuous acetic acid (the acid component of vinegar), water, and oxygen. The use of peracetic acid thus falls in the category of a processing aid by regulatory definition *(12)*, exhibiting no ongoing technical or functional effect in finished food products.

Along with the expanded use throughout the industry, recent regulatory updates have approved the use of peracetic acid at levels ten times higher than that specified in the original approval (e.g., FDA FCN 1495). Although regulatory authorities agree that peracetic acid and hydrogen peroxide do not persist on meat and poultry, their rate of degradation, which is the focus of the present study, has not been published/studied. The study design was to treat chicken and beef samples at a level of peracetic acid slightly above the current allowable maximum level of 2000 ppm, drain treated samples for incremental periods of time, and then recover and measure the residual levels of peracetic acid and hydrogen peroxide from the samples, allowing for calculation of decay kinetics of both chemical species. In addition to demonstrating the degradation of both species on either meat type, this study provides a time estimate of the zero-point residue levels for each chemical species (peracetic acid or hydrogen peroxide) for each type of meat tested. Such data are frequently used in the evaluation of finished food product safety and meeting certain regulatory authority requirements of negligible residue remaining on product for processing aid qualification.

MATERIALS AND METHODS

Fresh, non-frozen chicken drums (with skin and bone) and round steak samples were acquired at a local grocery store and used within 48 hours of purchase. Samples were stored at refrigerated temperatures until used. Chicken drums were used as purchased, beef samples were cut approximately in half, such that each chicken or beef sample tested was in the range of 50–100 g. Commercial peracetic acid (herein referred to as "commercial peracid") was used for each of the studies, with nominal equilibration levels of 15.2% peracetic acid and 11.2% hydrogen peroxide (Ecolab Inc. St. Paul, MN). All dip water used in this study was controlled at 5 grains per gallon hardness. Water was at room temperature (20–22°C) at time of use.

Antimicrobial solution preparation

Peracetic acid (PAA) dip solution was prepared by adding 10 kg of water to a clean, 20 l plastic pail, into which the commercial peracid was added and thoroughly mixed. The mixed solution was then analyzed using the suppressed peroxide iodometric method *(10)* for peracid and peroxide levels with 0.1 N thiosulfate titrant. The peracetic acid and hydrogen peroxide levels of the dip/immersion solution was verified before and after all testing to verify that the concentration of peracetic acid was between 2000 and 2300 ppm. For each test of meat immersion, ~600 ml of a large stock of immersion solution was added to a 1 l beaker, and more was added if needed to fully submerge the chicken or red meat sample. Immersion solutions were used once and discarded, so that the nominal peracetic acid solution used throughout the study was kept constant.

Meat preparation and antimicrobial application

Samples were manually placed into the peracetic acid dip solutions and agitated for 30 s, after which time they were aseptically removed with a plastic utensil and set on a rubber-coated metal rack for the set drain times. Whirl-

Pak bags were pre-filled with 205 g of de-ionized water to facilitate sufficient coating of the sample piece during the recovery process, and so that each rinse solution titration sample weighed at least 100 g. Individual meat samples were placed into the bags after set drain times had elapsed. The bags were sealed and manually shaken for 30–40 s, in such a way that the rinse solution completely covered the sample. The bags were then opened, the meat samples were removed and discarded, and the rinse solutions were analyzed for peracetic acid and peroxide levels.

Sampling and analysis

Both types of meat samples were tested in duplicate at each time point, and measurements were repeated on three separate days, for a total of n = 6 samples at each time point. For each meat sample, two separate titrations were performed on the same rinse solution, so that for each time point, 12 data points were obtained. Titrations used the same suppressed peroxide iodometric method *(10)*, optimized for sensitivity. Briefly, to a clean 250 mL Erlenmeyer flask with a Teflon-coated stir bar, one or two ice cubes were added to maintain peroxide suppression. The flask was tared on a balance, at least 100 g of the chicken or red meat rinse sample solution was poured into the flask from the Whirl-Pak bag, and the sample weight was recorded. The flask was placed onto a lighted stir plate; 2–4 ml each of glacial acetic acid (Ecolab Supply Chain, peracid grade), starch indicator (Ecolab Cat. No. 56869), and potassium iodide (10%, Ecolab Cat. No. 56867) were added while stirring. Reagents are added in excess, and exact volumes are not critical, as the measurement relies on the weight of the sample solution added to the flask for peracetic acid or peroxide quantitation. A change in the rinse solution color to blue indicated the presence of peracetic acid. The solution was titrated using 0.01 N thiosulfate standard (BDH, Cat. No. BDH7256, obtained through VWR Scientific), delivered in 200 µL increments (for short drain time samples, when the peracetic acid or peroxide levels were high), or 20 µL increments using an Eppendorf Multipette, Repeater Plus model (Cat. No. 2687194), fitted with a 1 ml pipette tip. Rapidity in the procedure is accomplished using this titrant addition method, allowing for distinguishable lightening of solution color with lower titrant volume deliveries, thus preventing overshooting of the titration endpoint. A color change from blue to white/clear which held for at least 10 s was considered a completed titration for peracetic acid.

Immediately following peracetic acid titration (or if no blue color was observed after addition of the first reagents), and on the same test solution, 2–4 mls each of 9N sulfuric acid (prepared from 50% stock, Ecolab Supply Chain, peracid grade) and oxygen catalyst (1.2% Molybdate (w/v), Ricca Chemical Company, Cat. No. 5190-1) were added to the flask and mixed for 3–5 minutes. A change in the solution color from white or colorless and back to dark blue indicated the presence of hydrogen peroxide. The solution was further titrated using the same 0.01 N thiosulfate standard, previously described, delivered in 200 µl or 20 µl increments.

Limits of detection (LOD) and quantitation (LOQ)

LODs and LOQs were determined for this titration method via visual observation of the colored indicator and changes imparted by titrant addition. LODs and LOQs were calculated based on potential observable color change, combined with the smallest titrant volume, a defined minimum sample weight, and the concentration of the titrant addition. For this study, a change in the coloration upon addition of the titrant 0.01 N thiosulfate was considered to be an indication of either species being present (depending on place in the titration procedure), and thus helps define the Limit of Detection. As the smallest titrant volume delivered was 20 µl, and the sample size of at least 100 g was maintained throughout, the LOD determination is defined according to the equation *(10)* used to measure the peracetic level:

$$((0.02 \text{ ml} * 0.01 \text{ N} * 38 * 100) / (100 \text{ g} * 1000))*10000 = 0.08 \text{ ppm}$$

where 0.02 ml is the minimum titrant volume, 0.01 N the thiosulfate concentration, 38 is the equivalence factor for peracetic acid (MW/2 to account for the titration reaction, which requires 2 molar electrons), 100 g is the weight of the sample, and 1000 converts the results, from milliequivalents to equivalents. The 100 factor in the numerator converts the result to a percent, and the 10,000 factor further converts the result to parts per million. For peroxide, the factor 38 is changed to 17, and the resulting LOD is 0.03 ppm. Limits of Quantitation were defined as 3 × these factors (0.24 ppm peracetic acid and 0.09 ppm hydrogen peroxide).

Data analysis

A response surface design of one factor (time) and two responses (residual analytes—hydrogen peroxide and peracetic acid) were used for this study. Two independent samples of meat were measured in duplicate via titration at each time point. The duplicate readings on each sample were averaged prior to statistical analysis. The entire experiment was run on three separate occasions and all the data combined in a single data set. This resulted in six samples at each time point for each type of meat.

Data were further processed to normalize the results based on the individual meat sample weights, such that an amount of peracetic acid or hydrogen peroxide was calculated and then divided by the sample weight. Error in the sample weight measurement was considered negligible and was not taken into account.

Plots of analyte vs time were generated for each analyte of interest on each type of meat. These plots revealed that the residues decayed according to classic exponential decay kinetics, and thus validated the use of a natural logarithmic treatment of the peracetic and peroxide content values. Linear regression was used to fit a straight line of the natural logs of the data to time. JMP Pro (ver. 13.1.0 (64-bit), SAS Institute Inc., Cary, NC) was used for the regression fit and to obtain the ANOVA output. The raw data contains zero values (or < LOD), causing the natural log to be undefined. To overcome this in our calculations and modeling, we have added 0.1 to all measured results in order to preserve work in the natural log scale and use our linear model. Therefore, our actual estimates in the inverse prediction are predicting when the remaining chemical level is 0.1 mg/kg. Four linear models were fit of the natural logarithms vs time—two types of meat (red meat and chicken) for each of two chemicals (peracetic and peroxide). The resulting ANOVA tables from the curve fitting are presented in *Table 2* and the fitted equations are found in *Table 3*. In all cases, the model fit was statistically significant.

Inverse prediction was used to determine the time point where the natural log reached -2.3026 (the natural log of 0.1). These predictions of the average expected response with their 95% confidence intervals for each chemical on each type of meat are provided in *Table 4*.

RESULTS

All results determined herein are specific to the equilibrium peracetic and peroxide levels of the commercial 15.2% peracetic acid, 11.2% hydrogen peroxide product; other peracetic acid providers may exhibit different equilibrium ratios, with different kinetics profiles. Data summaries for peracid/peroxide recovered residue levels at set drain time points are tabulated for chicken drum samples and for red meat samples in *Table 1*. For chicken drums, the data demonstrate both that the residual levels of peracetic acid steadily decreased over time and that experimentally no (or < LOD) peracetic acid was observed after 30 minutes of dry time. Hydrogen peroxide levels were also observed to reach a non-detectable level after 30 minutes. For red meat, the peracetic acid level was found to reach a non-detectable level at the 4-minute drain time, and a non-detectable level for hydrogen peroxide at the 16-minute drain time, indicating different rates of decay between the two chemicals.

The initial rapid drop in either peracetic or peroxide levels are characteristic of data that are considered to have an exponential decay pattern. Because of this, data were further processed by taking the natural log of the measured values for peracetic or peroxide content relative to sample weight, with the resulting plots generated and presented in *Figures 1 and 2*. If < LOD were to be considered 0, then the natural log of < LOD is undefined. To avoid this, 0.1 was added to all data. Therefore, our goal in the model fitting was to find at what time point the natural log reached 0.1 (or < LOD). A summary of all of these predictions with the 95% confidence intervals for the two sample types tested and each respective chemistry is presented in *Table 4*.

For poultry samples, interpolation of the PAA degradation curve predicts that the PAA level on poultry reaches 0.1 mg PAA/kg at 27.9 minutes. Hydrogen peroxide on poultry followed the same pattern, with the experimental finding that after 30 minutes, no residual peroxide was detected (< LOD, or 0.02 ppm or 0.42 mg/kg meat for a 100 g sample). By interpolation, the degradation curve predicts that the peroxide level on chicken reaches 0.1 mg H_2O_2 at 29.1 minutes.

For red meat, peracetic acid levels were experimentally determined to reach a non-detectable level at 4 minutes, and by interpolation at 3.5 minutes, again with close agreement between the experimental and calculated values, and within the error of the study results. Hydrogen

TABLE 1. Chicken drum and red meat peracid/peroxide recovered residue levels at set drain time points

Time Point (min)	mg PAA/ kg chicken	mg H_2O_2/ kg chicken	mg PAA/ kg red meat	mg H_2O_2/ kg red meat
0.5	Not Tested	Not Tested	12.0	23.0
1	15.8	12.9	4.7	16.1
2	10.5	7.7	1.2	10.4
2.75	Not Tested	Not Tested	0.2	4.6
4	5.8	4.4	< LOD	3.4
8	3.2	2.2	< LOD	0.5
16	0.4	0.7	< LOD	< LOD
30	< LOD	< LOD	Not Tested	Not Tested

TABLE 2. ANOVA tables*

Sample/Chemistry	Source	DF	Sum of Squares	Mean Square	F-Ratio	Prob > F
Chicken/PAA	Model	1	113.8417	113.842	477.66	< 0.0001
	Error	34	8.1033	0.238		
	C. Total	35	121.945			
Chicken/H_2O_2	Model	1	93.1790	93.179	595.84	< 0.0001
	Error	34	5.3170	0.156		
	C. Total	35	98.496			
Red Meat/PAA	Model	1	64.6244	64.624	477.66	< 0.0001
	Error	24	15.9720	0.666		
	C. Total	25	80.596			
Red Meat/ H_2O_2	Model	1	95.9089	95.909	108.98	< 0.0001
	Error	36	31.6826	0.880		
	C. Total	37	127.5915			

*0 from linear model fit, regressing ln (residual analyte/kg meat type) on time in minutes

TABLE 3. Fitted equations for Figures 1 and 2*

Sample/Chemistry	Equation	Figure
Chicken/PAA	Y = 2.58 – 0.175 * X	1
Chicken/ H_2O_2	Y = 2.30 – 0.158 * X	1
Red Meat/PAA	Y = 2.90 – 1.486 * X	2
Red Meat/H_2O_2	Y = 2.86 – 0.421 * X	2

*Based on the linear model fits. Y = LN (mg Residual / kg meat type + 0.1). X = Time (minutes).

TABLE 4. Inverse prediction summary table for PAA and H_2O_2*

Sample/Chemistry	Predicted time point (min)	Lower 95% CI	Upper 95% CI
Chicken/PAA	27.9	26.2	30.0
Chicken/H_2O_2	29.1	27.4	31.0
Red Meat/PAA	3.5	3.1	4.0
Red Meat/ H_2O_2	12.3	10.7	14.5

*prediction for what time point ln (0.1) is achieved

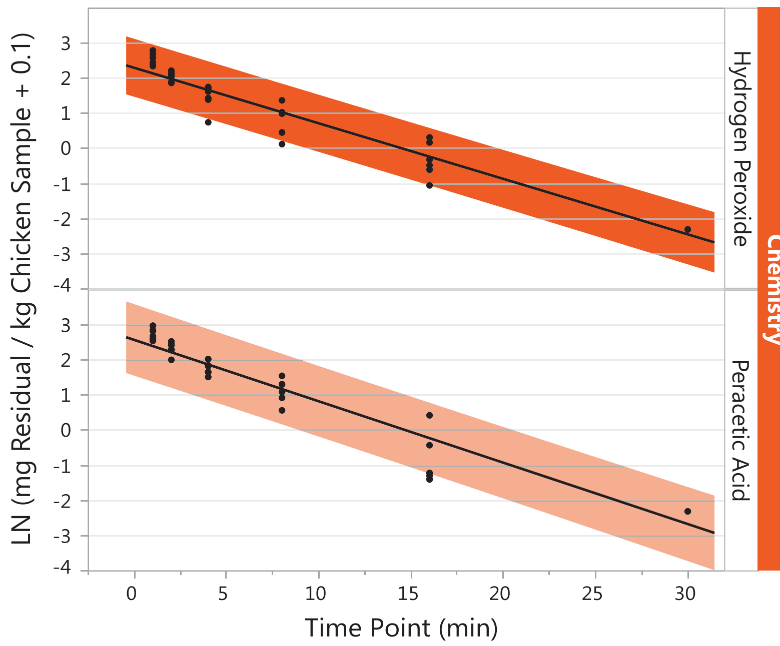

FIGURE 1. Residual hydrogen peroxide and peracetic acid recovered from chicken drums as a function of drain time. Shaded areas on either side of the regression curve fit indicate the 95% confidence intervals.

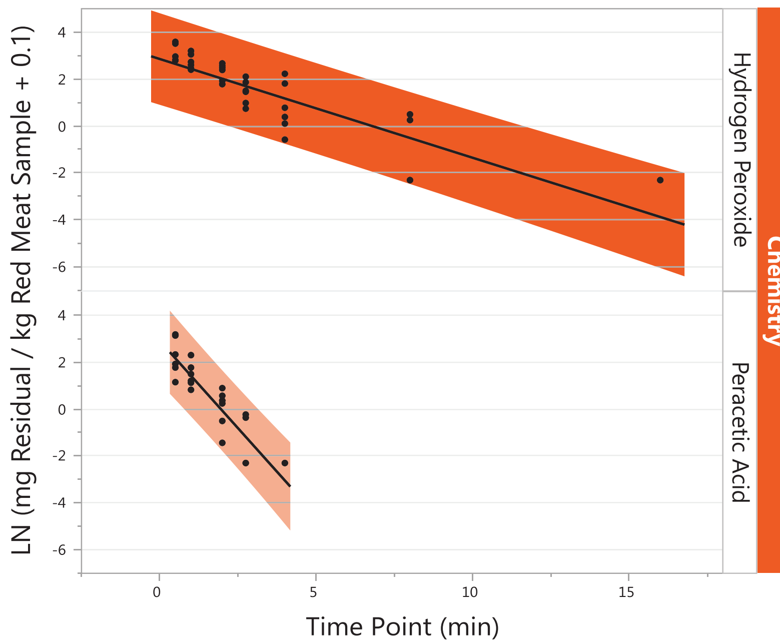

FIGURE 2. Residual hydrogen peroxide and peracetic acid on red meat as a function of drain time. Shaded areas on either side of the regression curve fit indicate the 95% confidence intervals.

peroxide levels were experimentally determined to reach a non-detectable at 16 minutes, and by interpolation at 12.3 minutes.

Samples that are subject to recovery of analytes of interest are subject not only to slight variations in the timing of the recovery process events but also to the potential for sample parts to become detached or solubilized in the recovery solution, and in turn a part of the measurement process. Overall, the tendency was for more variance in the data for the shorter drain times, in which the peracetic or peroxide levels were also at their highest. Sample to sample variations, then, are the more likely source of this larger variation, and not necessarily the result of the analytical system utilized throughout.

DISCUSSION

The current study investigated peracetic acid and hydrogen peroxide decay kinetics of chemicals as a function of time. Although the trends for both chemicals on each type of meat tested were in keeping with the expectation that lower levels of each would be measured as the time interval was increased, both the overall rates of decay as well as the species that decayed more rapidly differed markedly for each type of meat tested. A consideration of the types of meats and their composition helps to explain these differences.

The study results demonstrate decay kinetics with similar breakdown rates on the chicken drums. Considering the composition of the chicken drum, there exist large variations in the types of tissue present in the study, namely bone, skin, and muscle. Chicken skin is predominantly made up of fats and connective proteins, whereas red meat muscle tissue is predominantly iron-rich proteins, important for oxygen delivery in the living tissue *(1)*. The breakdown of peracetic acid and hydrogen peroxide are attributable to these proteins and other iron-containing compounds *(9)*.

Peracetic acids sold in the markets are equilibrium products, meaning that they contain some level of the precursors as well as peracetic acid. The ratios of peracetic acid to hydrogen peroxide are very likely different depending on the provider; therefore, the results presented here are unique to the specific intervention tested (commercial 15.2% peracetic acid, 11.2% hydrogen peroxide product). Although the level of peroxide in the concentrate (and therefore in the solution) was lower than that of the peracetic acid; the decay times to zero point predictions were fairly similar for both chemicals, with the peracetic acid reaching zero slightly before the peroxide. This finding suggests that the chicken tissue, and any oxidizable entities contained therein, have similar pathways for reactivity and breakdown of the peracetic and peroxide into its equilibrium precursors (acetic acid and water). As this study was conducted on commercial retail samples, there exists the possibility of increased breakdown kinetics for fresh-slaughter birds, as the tissue would be expected to have more blood, both as a result of the processing and within the meat tissue itself. Also, with the red meat samples, iron in the blood is very likely a causative factor for increased decay kinetics, as peracetic acid is sensitive to iron and catalytically degrades when iron is present in solution. The presence of blood also presents the possibility of presence of the catalase enzyme, which has been demonstrated to have unique specificity for catalytically degrading peroxide but not peracetic acid *(5)*. That either of these would be expected to be present at lower levels in the test samples indicates that the decay kinetics determined in these studies would be a worst-case scenario, especially with regard to study concentrations being slightly above the allowed level of 2000 ppm PAA, indicating that the true zero-point determinations are at shorter times in a processing facility. The magnitude of the difference is not expected to be large, likely on the order of minutes, as the observed decay times for the study samples were not extremely long to begin with, both chemicals having decayed to less than LOD levels within about 30 minutes.

Peracid and peroxide decay trends for red meat were observed to be much faster overall than those for chicken drums; however, the first to decay to the zero point was the peracetic acid, which dropped very rapidly below the LOD, within 5 minutes. Again, a consideration of the composition of the meat helps identify the most probable reasons for the rapid decay kinetics. Red meat is mostly comprised of muscle, with small, dense areas of fat and connective proteins throughout. Inherent to the muscle tissue are myoglobin and some hemoglobin *(3)*, which are at lower levels in chicken muscle. Both proteins have iron at their center, primarily for oxygen transmission, and this propensity for oxygen reactivity is the likely reason for the observed rapid decay of peracetic acid.

Red meat samples as tested were exposed to excessive peracetic acid levels by comparison to either common use levels or regulatory permitted use concentrations (which vary between 400 ppm and 2000 ppm PAA, at the time of this writing *(11)*). Exposure to such a high level of peracetic acid had notable effects on the color of the meat samples, turning the samples browner in color from their initial cherry red color. Peracetic acid is more commonly used in meat and poultry establishments at levels < 500–700 ppm, or ~ 25% of the level used in the study, and/or with shorter exposure times at higher PAA levels. For the lower exposure levels, the recovery and measurement of PAA and peroxide would pose challenges in both the capability to perform the recovery and analysis rapidly and the ability to detect lower levels of peracetic acid or peroxide.

This study demonstrates that peracetic acid and hydrogen peroxide rapidly decay below the LOD and LOQ levels within 30 minutes of immersion exposure. The data can be

used to reasonably predict decay times for poultry and red meat exposure, and further demonstrates that peracetic acid and peroxide residuals have no functional effect on finished meat products and would be negligible at time of retail sale or consumption.

Chemical species in the processing aid used in this study decayed to levels below their LOQ and LOD. The finding could have implications for methodologies used in determining microbial load following treatment: for poultry sampling, it suggests that instead of introducing a neutralizing agent in the recovery broth, simply waiting a prescribed amount of time would assure that there was no interference from this particular processing aid. This study also supports previous research by Gamble et al. *(4)* by providing clarity in tracking the residual PAA level. In their study, simulated process testing for sanitizer efficacy from carryover was found to be efficacious for PAA at drain times of 0 and 1 minutes, but not at 5 minutes. As demonstrated here, the PAA level (and very likely its efficacy) will diminish given a sufficient waiting period before sampling. Similar testing on the other oxidative and non-oxidative processing aids would need to demonstrate a similar pattern of reaching a zero-point determination to warrant a similar claim of not requiring a neutralization agent in demonstrating intervention efficacy.

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