

Persistency of Bromine Residuals on Carcass Surfaces With Treatments of DBDMH and HOBr

Background

Bacteria levels on beef carcasses must be controlled during the slaughtering process at packaging plants. After the bovine are slaughtered, the skin is removed from the carcass and then cut in half exposing a layer of adipose tissue, which covers the carcass. Adipose tissue is commonly known as fat. The chemical composition of adipose tissue is composed mostly of lipids in the form of triglyceride, unlike the nitrogen organic material muscle is composed of. After a carcass has been halved, the two halves of the carcass, covered in adipose tissue, are sanitized. The carcass is sprayed using boiling water or a chemical treatment to kill bacteria on the exposed surfaces. Chemical treatments are favored substantially more than water treatments in packaging plants due to the fact that hot water partially cooks the surface of the meat and is not cost efficient, but leaves no antimicrobial effects on the surfaces.

There are several chemistries available for the use of a carcass washing sanitizer. This study compares three different chemistries: peracetic acid, 1,3-Dibromo-5,5-dimethylhydantoin (DBDMH), and hypobromous acid (HOBr) made from a solution of hydrogen bromide and sodium hypochlorite that is mixed on-site. Peracetic acid (PAA) is commonly utilized in carcass washing facilities as a sanitizer. It provides excellent initial sanitizing qualities then decomposes rapidly to acetate and water, which have no sanitizing properties. Peracetic acid only provides a large initial reduction in bacteria. Previous studies have established that continual sanitizing qualities do not persist on meat due to the nitrogen containing organic material in the muscle. After one minute contact time, there is no measureable PAA residual remaining. There have been speculations as to the persistency of bromine on carcasses. This study was completed to determine, (1), the persistency of bromine residuals on the outer layer of carcasses and trim, and (2), if the persistency of PAA on adipose tissue is comparable to bromine.

Methodology

This study would take place after the removal of the skin and halving of the carcass. The carcasses are vertically disposed during treatment. Figure 1 illustrates the carcasses after skinned and halved.

Chemical analysis for PAA and available bromine was performed using the modified DPD analysis technique, which is included as Attachment #1.

FIGURE 1



The light coloring covering the majority of the surface of the carcass is the adipose layer. Once the carcasses are cut in half, internal organs and ribs are exposed. The sanitizing treatment is applied to all exposed surfaces. There is also muscle meat exposed at this point of the slaughtering process. The majority of the carcass exterior is adipose tissue; therefore the chemical treatments in this study were applied to a combination of adipose tissue and muscle meat.

DBDMH, the current bromine chemistry, and HOBr, a new liquid bromine chemistry, which has been assigned an FCN #944 by the FDA/FSIS, were incorporated in this study to determine the persistency of bromine residuals on adipose tissue and to compare the persistency of bromine with peracetic acid. Strips of adipose tissue were used to simulate the outer layer of beef carcasses. The adipose tissue was cut into six sections of equal size and weight (for each challenge). The pieces were vertically disposed so excess solution was allowed to drip from the surface, simulating the process in packing plants. This is illustrated in Figure 2 and 3.

FIGURE 2



FIGURE 3



The front surface of each piece was spayed 10 times with 300 ppm as bromine (from DBDMH and HOBr activated solution) and 220 ppm as PAA (from Perasan MP2). The pieces were then rinsed with a known weight of deionized water to measure the halogen residual remaining on the tissue using the modified DPD Colorimeter method (Appendix A). This was repeated at different time intervals until the halogen residual was not measureable. Figure 4 illustrates the rinsing of an adipose section to remove any halogen remaining on the tissue.

FIGURE 4



Table 1 displays the initial weights and contact times used for the DBDMH treatment. Six sections of adipose tissue were used, weighing between 20.18 g and 20.54 g. The contact time ranged from 1-60 minutes.

TABLE 1 DBDMH: 300 ppm as Br₂

Adipose Tissue Sections	Time (min)	Initial Wt. Of Adipose Tissue Sections (g)
1	1	20.54
2	5	20.52
3	10	20.32
4	15	20.22
5	30	20.36
6	60	20.18

Table 2 represents the initial weights and contact times used when HOBr activated solution was the treatment. The weights of each adipose tissue section ranged from 19.74 g to 20.94 g. The contact time ranged from 1-60 minutes.

TABLE 2 HOBr Activated Solution: 300 ppm as HOBr

Adipose Tissue Sections	Time (min)	Initial Wt. Of Adipose Tissue Sections (g)
1	1	20.26
2	5	20.22
3	10	20.18
4	15	20.26
5	30	19.74
6	60	20.94

Table 3 represents the initial weights and contact times used when peracetic acid was used to treat the adipose tissue. The weights of each adipose tissue section ranged from 22.52 g to 20.84 g. The contact time ranged 1-5 minutes.

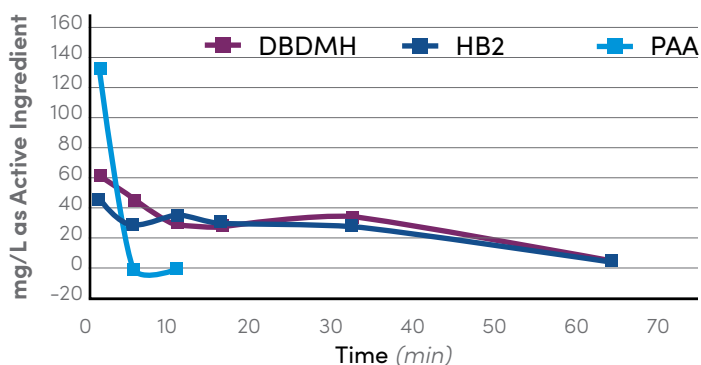
TABLE 3 PAA (220ppm as PAA)

Pieces	Time (min)	Initial Wt. Of Adipose Tissue Sections (g)
1	1	20.52
2	5	20.78
3	10	20.84

Graph 1 illustrates the halogen residual remaining on the sections of adipose tissue over 60 minutes. After a 300 ppm available bromine wash for each of the bromine-based products, the DBDMH treatment had 61.20 ppm as bromine residual remaining after a contact time of 1 minute. After 60 minutes, there was less than 4.16 ppm as bromine remaining. 1 minute after the HOBr treatment, 44.94 ppm as bromine residual remained. After 60 minutes, there was less than 4.42 ppm as bromine remaining. DBDMH had an expected higher bromine residual throughout the study due to its more stable DMH chemistry. The study was terminated after 60 minutes because the rinse solution no longer developed a pink hue when the DPD powder was added and the reading was at the lowest range of the colorimeter. The bromine residual remained on the adipose tissue over the course of an hour. The persistency of the bromine allowed for longer contact time; effectively elongating the sanitation qualities of the bromine chemistries, DBDMH and HOBr activated solutions. The peracetic acid had a high recovery at 1 minute contact time, but degraded before 5 minutes. There was 133.09 ppm PAA recovered at one minute, but no PAA recovered at 5 or 10

minutes. PAA reacts similarly on adipose tissue as on meat, with a large initial reduction, but minimal or no sanitizing qualities after 1 minute.

GRAPH 1 Persistency of HOBr, DBDMH, and PAA on Adipose Tissue



Conclusion

After completion of this study, it can be concluded that bromine persists longer than peracetic acid on the surface of meat carcasses. Peracetic acid degrades into compounds that do not provide any sanitizing properties, therefore peracetic acid only provides a substantial initial reduction of bacteria, but provides little or no sanitizing qualities after 1 minute. Bromine residuals were measured up to an hour after treatment on the adipose tissue. Even though the concentration of bromine residual was minimal, its presence on the surface provides extended sanitizing qualities superseding that of peracetic acid. After 1 hour, the concentration of bromine residual was ineffective. Using linear log regression coefficient analysis, it is estimated that the HOBr solutions are completely degraded to bromide ion within about 72 minutes after application of a 300 ppm solution.

The initial lower recovery and faster decay of HOBr-activated solutions can only be explained by the inherent differences of DBDMH and hypobromous acid (HOBr). Since HOBr has a higher oxidation potential than DBDMH, the oxidation process would naturally consume more of the active HOBr. DBDMH has been shown to be a hypobromous acid-DMH complex, with lower oxidation capacity than free HOBr. Thus, the HOBr (HOBr) would be expected to react/ degrade more quickly initially. This would explain why the HOBr microbial challenge tests done by this laboratory indicate the consistent but measurably improved efficacy of HOBr (hypobromous acid) for short-term microbial challenge tests performed and reported in other efficacy studies.

For a period of 10-60 minutes after application of the processing aids, there appears to be no difference in the over-all decay rate of the total bromine residual for either DBDMH or HOBr (hypobromous acid). However, it is notable that 30 minutes after application of either FCS, a residual of available bromine is still measurable on the adipose tissue surface. Although a decay regression coefficient analysis was not performed on DBDMH, it is suspected to decay at a slower rate than HOBr, again due to its more stable molecular activity and structure.

TITLE:

HOB_r: PROCEDURE FOR VALIDATION OF BROMINE AND CHLORINE for PROCESS EQUIPMENT (Modified DPD Method)

PURPOSE:

This document is to be used by any lab personnel to verify the balance of mixtures of activated HOBr solutions using the HACH DR/890 Colorimeter or equivalent. It is recommended that this procedure be followed at least on a weekly basis (or at each plant option, more frequently) because this method can very accurately distinguish between hypobromous acid and bleach. This distinction is quite important, because most subsequent automated monitoring equipment cannot distinguish bromine from chlorine. Once the efficiency has been established, the option of using a HOBr drop test kit for routine testing can be used by personnel on the floor to reassure that the bromine concentration is at the appropriate level.

EQUIPMENT / REAGENTS:

- HACH DR/890 Colorimeter- Catalog number 48470-00 (or equivalent)
- or: HACH Colorimeter 2 Catalog number 58700-00 (or equivalent)
- De-ionized or reversed osmosis water
- Glycine solution (Enviro Tech #D-10015-03) or equivalent. Glycine should be refrigerated. It may also be frozen and thawed before use.
- DPD FREE Chlorine Reagent Pillow Packets (for 10 mL)- HACH # 21055-69
- Potassium Iodide Crystals (KI) – SHAPE Products catalog #8118 (or equivalent)

PROCEDURE:

Use this section daily or weekly (optional) to ensure the halogen present is bromine.

Before testing make sure the instrument is in the low (LO) range mode by checking that the display reads to the hundredths (0.00).

1. Make an appropriate dilution. For example, (testing a theoretical 300 ppm as Br₂ solution) weigh/measure 97 mls RO water, add exactly 1.00g or ml of HOBR solution and then add 2 ml of glycine solution.
2. Fill both 10 ml sample cells with 10 mls of the water sample. Designate one of these to be the blank and the other to be the prepared sample. Make sure the cells are not wet and they are free of fingerprints or smudges.



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Tina Rodrigues

Lab Manager

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Revised Section(s):
N/A until a revision is made

Without a yellow control stamp to the right of this statement, this procedure is a draft. A draft or an uncontrolled copy cannot be used to manage a process or task.

3. Cap the blank cell and place it in the cell holder with the diamond mark facing you. Cover the cell compartment and press ZERO. The instrument will display 0.00. Remove the blank.
4. Add the contents of one DPD FREE Chlorine pillow packet to the prepared sample. Cap and shake vigorously. A pink color will develop.
5. Place the sample cell in the compartment with the diamond mark facing you, close the cover and press READ.
6. The instrument display will show "--" followed by the results in ppm total chlorine. Call this reading "B".
7. Note: if the instrument reads a blinking 3.67, the sample concentration is too high and needs to be diluted further before resuming with step #8.
8. Remove the sample cell from the compartment and add a small amount of KI crystals (two crystals) to the sample cell still containing the sample. Vigorously shake for 15 seconds, this step allows the glycine-combined chlorine to react with the KI.
9. Place the sample cell in the compartment with the diamond mark facing you, close the cover and press READ. This reading is ppm Total Halogen. Call this reading TH.

Ideally, results from step 6 and step 9 should be the same, $\pm 5\%$:
 $TH = B$. If not, then some of the halogen is present as Chlorine (Cl)*: Therefore,

$$Cl = TH - B$$

*If this is the case, let the solution mix longer and repeat the test. Or it may be necessary to adjust the chemical mixing ratio of the HOBR & bleach blending device.

Calculations:

$$\text{Available Bromine} = TH - B \text{ reading} \times 2.25 \times 100$$

(dilution factor)

**Reminders:

- (1) Multiply colorimeter reading by dilution factor used.
- (2) Sample cell and cell cap must be washed thoroughly between replicates to remove KI residues, which will interfere with the accuracy of the subsequent samples.