ENVIROTECH

HB2

EFFICACY REPORT

Persistence of High Concentrations (900 ppm) of Hypobromous Acid Residuals on Treated Beef Tissue Surfaces

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12/07/2010

Background

Bacteria levels on beef carcasses must be controlled during processing. After the bovine are slaughtered, the hide is removed from the carcass which is then eviscerated and cut in half exposing adipose and muscle meat tissue. Adipose tissue is commonly known as fat. The chemical composition of adipose tissue are mostly lipids, in the form of triglycerides. Muscle meat comprises the proteinaceous, nitrogen-rich organic material. After the carcass has been halved, all the exposed surfaces are sanitized using a spray of either hot water or sprays of a sanitizing chemical, or a combination of the two. Chemical treatments are favored over hot water treatments because the heat partially cooks and decolors the surface muscle meat, as well as dissolving some of the outer fat.

There are several chemistries available as a carcass washing sanitizer. This study scrutinizes the latest chemical treatment option for processors, hypobromous acid (HOBr), from a precursor compound known as aqueous hydrogen bromide, which is activated into hypobomous acid by using a hypochlorite source, such as sodium hypochlorite (bleach).

This study was performed to examine the stability of HOBr in the presence of beef adipose and muscle meat tissues when applied at elevated levels. Currently, FCN 944 permits HOBr to be applied to meat carcasses at levels up to 300 ppm as Br2. Several beef processing companies have expressed an interest in gaining the approval for a level that is 3 times this concentration, and approaches the concentration and efficacy equivalence of acidified sodium chlorite applied to animal carcasses. Therefore, various studies were performed using 900 ppm HOBr (as Br2).

A food contact substance that has a "lasting technical effect" requires ingredient labeling on the package of meat products. Typically, the FSIS considers a lasting technical effect to be about 24 hrs. after application. Therefore, in order to establish the timeline for the residual effect of hypobromous acid at higher concentrations (if any), the following study was performed. Microbiological evaluations were not performed because the persistence of hypobromous acid, which is the active antimicrobial compound, did not last beyond 5 hrs in any of the challenge testing.

Methodology

The Decay of HOBr on Lean and Adipose Tissue

This study was designed to model worst-case scenario conditions and employed submerged beef tissue at a concentration of HOBr three times greater than currently allowed under FCN 944 allowed.

In this study, the volume of HOBr solution to the surface area of beef tissue was calculated to be 30 times greater than that of the amount of solution on beef tissue that had been sprayed. In addition, submersion in the challenge FCS would be more efficient in terms of complete coverage of the FCS on the meat substrate, as compared to spray or fogging methods.

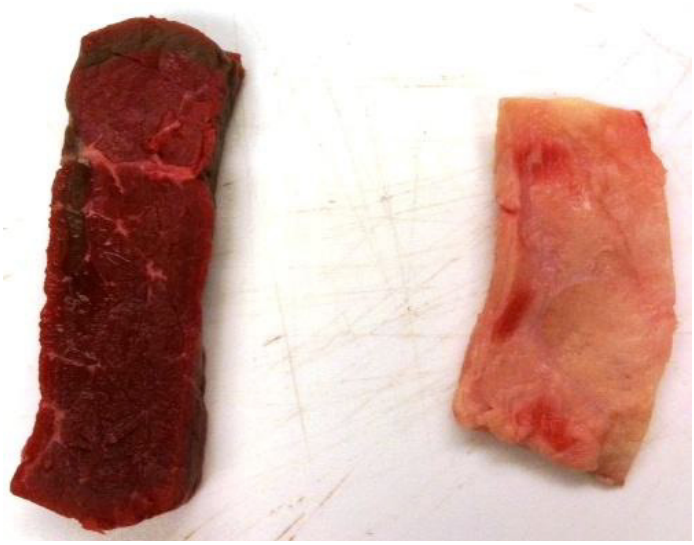
A 900 ppm as Br2 solution of HOBr was prepared in city water (1260 g) by adding HB2, the commercial grade hydrogen bromide 24% (2.94 g), and bleach 10.32% as Cl2 (5.36 g). The activated solution was tested using the modified DPD (glycine) method to speciate the amount of available bromine and chlorine in the solution. In this case, all the halogen utilized was present as HOBr, and excess hypochlorous acid (HOCl) was not detected. Table 1 displays the weights and concentrations of the various ingredients of the challenge solution.

TABLE 1

Raw Materials	Actual Wt. (g)	Concentration ppm Cl_2 bleach	Concentration ppm as Br_2
City Water	1260		
24% HBr	2.94	0	967.5
Bleach (10.32% as Cl2)	5.36		

Illustrated in Figure 1 is a representative strip of lean meat and adipose tissue used to simulate the outer layer of beef carcasses. Ten pieces of London broil were cut to have a average surface area of approx. 140.5 cm^2 (dimensions 13 x 3.5 x 1.5). The meat pieces were placed into small containers with sufficient HOBr solution (150 mL) to be completely submerged. This is illustrated in Figure 2. The average volume of the solution per unit surface area was 1.07 mL/cm^2.

IMAGE 1



Thereafter, pieces of adipose tissue were cut to have an average surface area of approximately 102.3 cm^2 (dimensions 9.5 x 4.5 x 0.6). The pieces were placed into small containers with sufficient HOBr solution (100 mL) to submerge them. This is illustrated in Figure 3. The average volume of the solution per unit surface area was 0.98 mL/cm^2.

IMAGE 2 (MEAT)

IMAGE 3 (ADIPOSE)

The solutions in contact with the lean muscle meat and the adipose tissue were sampled over time to monitor the decay profile of the HOBr. The decay profile on the surface of the meat would be identical to the profile of the solution chemistry, as indicated by the obvious color of the solutions. The modified DPD Colorimeter method was used. Initially, a serial dilution was prepared using deionized water to assure the level of HOBr in the solution was within range of the colorimeter. The study was terminated when the HOBr level was close to the detection limit of the method

Results and discussion

The Decay of HOBr on Lean and Adipose Tissue

Table 2 displays the average weight, surface area and volume of solution per unit of surface area for the submerged pieces of lean meat.

TABLE 2 (AVERAGES)

Weight of Meat	80.92 g
Surface Area of Meat	140.5 cm^2
Volume of HB2 Activated Solution	150.5 mL
Volume Normalized to Surface Area	1.07 mL/cm^2

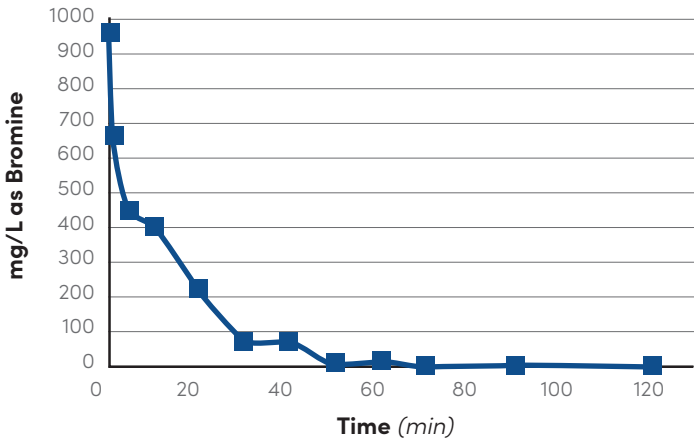
Table 3 displays the average weight, surface area and volume of solution per unit surface area for the submerged pieces of adipose tissue.

TABLE 3 (AVERAGES)

Weight of Adipose Tissue	27.00 g
Surface Area of Adipose Tissue	102.3 cm^2
Volume of HB2 Activated Solution	100.6 mL
Volume Normalized to Surface Area	0.98 mL/cm^2

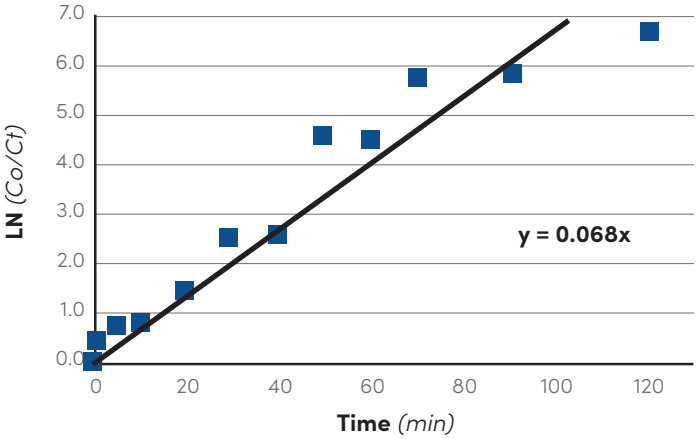
Graph 1 illustrates the instability of HOBr (expressed as Br2) in contact with lean meat. The initial concentration of the solution was 967.5 ppm as Br_2. After 1 minute, only 666 ppm as Br2 was remaining. After 120 minutes the study was terminated due to the rapid depletion of HOBr to only 1.13 ppm as Br2.

GRAPH 1 Stability of Hypobromous Acid in the Presence of Lean Meat (Worst Case Senario)



Graph 2 uses the data of Graph 1 for determination of the kinetic decay constants. When the results are plotted as the natural log of the ratio of the initial concentration of HOBr to the concentration at time 't' as a function of time, there is a straight-line relationship. Linear regression yielded a slope of 0.068. This indicates that the HOBr solution decayed with a rate constant of 0.068 min^{-1}, corresponding to a half-life of 11.34 minutes. The correlation coefficient, R^2 value calculated was 0.9355. Thus, the decay corresponds very closely to a pseudo first-order mechanism (perfect first order, $R^2 = 1$)

GRAPH 2 Decay Kinetics of Hypobromous Acid (expressed as Br_2) in the Presence of Lean Meat



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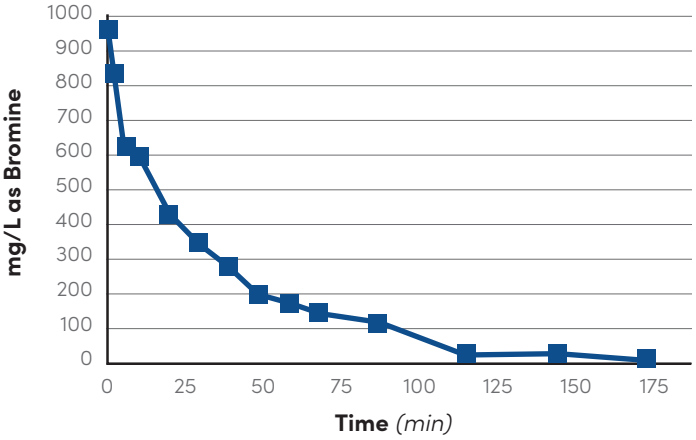
Graph 3: illustrates the instability of HOBr (expressed as available Br_2) in contact with adipose tissue. The initial concentration of the solution was 967.5 ppm as Br_2. After 1.5 minutes, 837 ppm as Br_2 was present on the solution. The level dropped below 200 ppm as Br_2 after 50 minutes. After 3 hours the study was terminated due to the depletion of HOBr to only 5.63 ppm as Br_2.

GRAPH 3 Stability of Hypobromous Acid in the Presence of Adipose Tissue (Worst Case Senario)



Graph 4 uses the data of Graph 3 for determination of the kinetic decay constants. When the results are plotted as the natural log of the ratio of the initial concentration of HOBr to the concentration at time t, as a function of time there is a straight-line relationship. Linear regression yielded a slope of 0.0283. This indicates that the HOBr solution decayed with a rate constant of 0.0283 min^{-1} corresponding to a half life of 25.62 minutes. The correlation coefficient, R^2 value calculated was 0.9882. Thus, the decay corresponds very closely to a pseudo first-order mechanism (perfect first order, R^2 =1).

GRAPH 4 Decay Kinetics of Hypobromous Acid (expressed as Br_2) in the Presence of Adipose Tissue

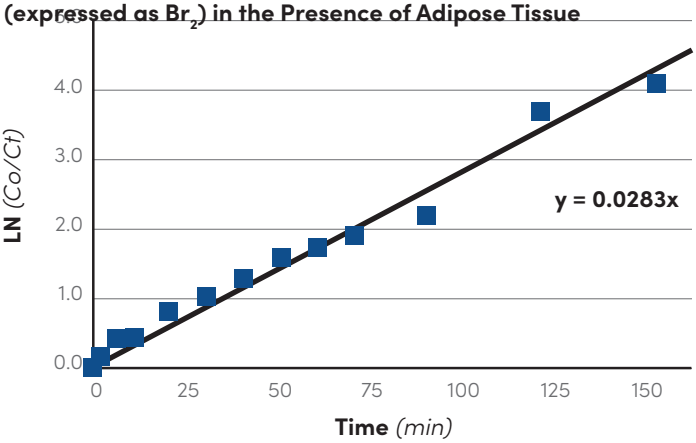


Table 4 displays the half-life for HOBr when in contact with lean meat and adipose tissue. The half-life of the halogen when in contact with lean meat was approximately 2 times less than the half-life of the halogen when in contact with adipose tissue.

TABLE 4

Challenge	Half-Life (min)
HOBr in Contact with Lean Meat	11.34
HOBr in Contact with Adipose Tissue	25.85

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Conclusions

- Hypobromous acid is highly unstable in the presence of both beef muscle meat and adipose tissue. It breaks down more rapidly in the presence of proteinaceous organic nitrogen material (muscle meat), than the primary triglyceride composition of adipose (lipid) tissue. Even at the initial high concentration of around 900 ppm (as available Br2), HOBr decays rapidly and with pseudo-first order decays constants on both lean and adipose tissue. Thus, regardless of the concentration of HOBr applied, there is no possibility that a HOBr residual would persist on any treated product by the time it reached the consumer.
- The present study modeled a worst-case scenario condition. The volume of HOBr solution per surface area of exposed animal tissue was calculated to be approximately 30 times greater than the volume of HOBr solution retained on a sprayed beef carcass. [The surface area of a beef carcass is approximated to be 45 ft2]. Thus, the current study would be termed "worst case scenario" for a commercial setting, in relation to the amount of chemical applied to the meat product per surface area volume.
- Even in contact with a volume of HOBr solution that is about 30 times higher than that which contacts sprayed beef tissue, the active halogen is quickly depleted. The half-life of HOBr on the surface of the lean muscle meat is approximately two and one-half times less than a HOBr solution that contacts adipose tissue. Under the experimental conditions employed in this study, the half-life of HOBr was 11.34 minutes on lean muscle meat and 25.85 minutes on adipose tissue.
- The modified DPD methodology was used to determine the active oxidative species of hypobromous acid and/or hypochlorous acid. The DPD method has been the standard test methodology used to determine chlorine (hypochlorous acid) and chlorine dioxide for many years. It should be noted that the standard test cannot normally differentiate between halogen species, such as chlorine, bromine and iodine. This applicant uses the modified DPD methodology utilizing glycine, which was originally developed for the determination of chlorine dioxide. Glycine is a simple amino acid that efficiently binds with and neutralizes hypochlorous acid but does not affect hypobromous acid during these measurement timelines. This modified DPD methodology is further explained in another scientific validation report by the current authors.