Evaluation of Solubilized Proteins as an Alternative to Phosphates for Meat Enhancement

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Project Summary

Background
Postmortem treatments are utilized by the beef industry to improve consumer eating experiences and perception of beef products. The use of phosphates to improve tenderness and juiciness and control oxidation in beef is well established. However, phosphates are additives that cause health concerns for select consumers, primarily those suffering from chronic kidney disease. As a result, the beef industry had an interest in searching out alternative techniques that maximize the health benefits of beef for all consumers while providing consumers with a good eating experience. The objective of this project was to determine whether or not injection of solubilized meat proteins can perform similarly or better than injections using a phosphate-based enhancement solution.

Methodology

Phase 1
Ten paired beef strip loins were randomly selected from USDA Select grade carcasses following a 48-hour chill. Strip loins were vacuum packaged and stored overnight. The following day, initial (green) weight of each subprimal was recorded and each paired subprimal was randomly injected with either the control (phosphate) or treatment (solubilized protein) solution at 4°C using a stitch pump enhancer calibrated to inject at 105% of the recorded green weight. Needles penetrated the top of the subprimal to 5.08 cm from the bottom to deliver the enhancement solution. For the treatment injection, an aqueous solution containing 1% Herbalox seasoning Type HT-W and 3.6% sodium chloride was first injected at 5% green weight into the subprimals. Then, a second injection at 5% green weight was made using prepared solubilized protein. The phosphate-based solution consisted of 4.5% phosphate, 3.6% sodium chloride, 90.9% water, and 1% Herbalox Seasoning Type HT-W. Two injections at 5% were conducted to mimic the double injection utilized with the solubilized protein-treated samples.

Treated subprimals were fabricated 30 minutes after injection into 2.54 cm thick steaks and steaks were individually placed into plastic trays with absorbent pads and packaged in a modified atmosphere (80% oxygen & 20% carbon dioxide). Packaged steaks were placed in boxes and put in a dark room at 4°C for 4 days to simulate transportation to retail stores. They were then transferred to a retail case at 4°C for the remainder of the study, days 5-11. Once in the retail display case, steaks were color scored each day until day 10. Two steaks were randomly selected from each treatment on days 5, 7, 9, and 11. One steak was used to measure retail case purge, cook yield and Warner-Bratzler shear force (WBSF) analysis. The other steak was used for sensory analysis. On days 5 and 9, a third steak was randomly selected for aerobic plate count (APC) and 2-thiobarbituric acid reactive substances (TBARS) analysis.

Phase 2
The purpose of Phase 2 was to determine which injection solution should be further investigated in Phase 3. This phase of the project evaluated the phosphate injection as in Phase 1 and the injection of solubilized protein also as described in phase 1 with the following modification. The first injection at 5% green weight contained 6.2% salt and 2% Herbalox. The second injection at 5% green weight remained the same and contained solubilized protein, pH 2.5. Both of these injections were used as references. Three additional treatments were investigated. A phosphoric acid injection
(pH 2.5) and an ammonium hydroxide injection (pH 11, ~1%) containing the same amount of salt and Herbalox as the phosphate injection solution. In addition, a higher protein injection solution was also evaluated containing 1:5 as opposed to 1:9 solubilized protein. Salt and Herbalox remained at the same concentration as indicated above. The design for the Phase 2 study involved injection of just two subprimals per treatment (2x5). Subprimals were injected, cut, packaged, and stored in the dark for 4 days as in the phase 1 study. Steaks were placed in retail display and were evaluated for selected parameters on day 5 (day 0 retail display) and day 12 (day 7 retail display). On day 5, parameters measured were purge, cook yield, APC, WBSF, TBARS, pH, and sensory. On day 12, TBARS, APC and pH were measured. The pH of steaks was also measured just prior and after injection.

Phase 3
Ten paired beef strip loins were randomly selected from USDA Select grade carcasses following a 48-hour chill. Strip loins were vacuum packaged and stored overnight. The following day, initial (green) weight of each subprimal was recorded and each paired subprimal was randomly injected with either a phosphate or an ammonium hydroxide based solution at 4°C using a stitch pump enhancer calibrated to inject at 110% of the recorded initial weight. For the ammonium hydroxide solution, an aqueous solution containing 1% Herbalox seasoning type HT-W and 3.6% sodium chloride adjusted to pH 10 using FCC grade ammonium hydroxide was injected into subprimals at 110% of green weight. The phosphate solution was prepared with 4.5% phosphate, 3.6% sodium chloride, and 1% Herbalox seasoning type HT-W. This solution was injected at 110% green weight.

Treated subprimals were fabricated 30 minutes after injection into 2.54 cm thick steaks and steaks were individually placed into plastic trays with absorbent pads and packaged in a modified atmosphere (80% oxygen & 20% carbon dioxide). Packaged steaks were placed in boxes and put in a dark room at 4°C for 4 days to simulate transportation to retail stores. They were then transferred to a retail case at 4°C for the remaining two weeks of the study. Three steaks were randomly selected from each treatment on days 5 (day 0 retail display), 12 (day 7 retail display), and 19 (day 14 retail display). One steak was used to measure retail case purge, cook loss, color and WBSF. A second steak was used for retail case purge, cook loss, color and sensory analysis. The third steak was selected for APC, anaerobic plate count, proximate analysis, and TBARS analysis.

Findings

Phase 1
Enhancement increased moisture content and lowered fat content of raw steaks and protein enhancement increased protein content. In the cooked steak, phosphate enhancement improved moisture content by about 2%. Protein-enhanced steaks began to differentiate in protein content by 5% at day 7 and 2% at day 9. For lean color, the moderately bright cherry red color was apparent only on the first day of display for protein-enhanced steaks. Phosphate-enhanced steaks stayed moderately bright cherry red for 2 days of display. Steaks from both treatments by the final day of the study were still slightly dark red. In general, phosphate-enhanced steaks performed better with respect to fat discoloration than protein-enhanced steaks. Protein-enhanced steaks were able to retain the red color as well as the phosphate-enhanced steaks. Overall, percent discoloration of enhanced steaks from this project was 1% to 10%.

At day 5, levels of APC bacteria were not significantly different and bacterial loads were approximately 4 logs. By day 9, steaks treated with the protein enhancement solution were 2 logs
higher in APC than phosphate-treated steaks. All values were within the acceptable limit for steaks. The phosphate treatment average for both days 5 and 9 was below 1.0 mg/kg. The protein enhancement at day 5 and 9 was already over the detectable limit, thus indicating that the protein enhancement did not perform as well. At day 5, the initial purge loss was significantly lower for phosphate-enhanced steaks than protein-enhanced steaks. For day 5, cook yield for phosphate and protein varied by 2% at 26% and 28%, respectively. This trend stayed consistent throughout the study with phosphate-enhanced steaks always having the lower or better percent cook yield.

Phosphate-enhanced steaks performed better than the protein-enhanced steaks but both samples fell into the WBSF category of tender (<4.5 kg). For the phosphate-enhanced steaks, the lowest WBSF values were recorded on day 9 of the study at 2.99 kg. The lowest WBSF values for the protein-enhanced steaks were achieved on day 5 of the study at 3.91 kg. Sensory panelists evaluated phosphate-enhanced steaks as being significantly more tender and juicier, containing less connective tissue, and being, overall, more acceptable. Although phosphate-enhanced steaks were significantly juicier, both treatments were categorized by panelists as slightly juicy. Connective tissue values were reported as slightly abundant for phosphate-enhanced steaks and moderately to slightly abundant for protein-enhanced steaks. Phosphate-enhanced steaks received an overall score of slightly desirable while the protein-enhanced steaks received a score of acceptable.

Phase 2
Results from this study indicated that increasing the protein content of the solubilized protein injection from 1:9 to 1:5 reduced purge and cook loss. Sensory and WBSF values were also improved; however, there was still a significant amount of purge. Phosphoric acid injections outperformed all other injection treatments in terms of sensory evaluation. WBSF for this treatment was also lower than all other treatments and only the phosphate treatment had better purge loss. The ammonium hydroxide injection treatment was generally as good, or better, than the phosphate injection and only the phosphoric acid injection slightly out preformed it in terms of sensory analysis. Purge loss for ammonium hydroxide was higher than both phosphate and phosphoric acid, but lower than either protein injection while cook loss was essentially the same as the phosphate injection. The only parameter in which the ammonium hydroxide solution injection did not perform as well as the phosphate injection was TBARS. TBARS values for phosphate injections were half that of all other treatments.

Taking these results into account, it was determined that the ammonium hydroxide injection was the most promising treatment tested. As a result, a phase 3 study was designed to be more cost effective utilizing ammonium hydroxide at pH 10 instead of pH 11 (less ammonium hydroxide required).

Phase 3
The target percent pump weight was 110% of the initial weight for the enhancement solutions. The ammonium hydroxide treatment was lower in pH than the phosphate treatment. Final meat pH was significantly lower as a result of the 1 log decrease in solution alkalinity from pH 11 in Phase 2 to pH 10 in Phase 3. Proximate analysis was performed on days 5 (0 day retail display), 12 (7 day retail display) and 19 (14 day retail display). No significant for fat, ash and moisture content between treatments was observed. However, the percent protein in steaks treated with ammonium hydroxide was higher than those treated with phosphate and this can likely be attributed to increased purge.

TBARS content was lower for phosphate injections. Over time, lipid oxidation increased. However, all values were below the threshold value of 1 mg maldonaldehyde (MDA)/kg of fresh meat.
meat for the duration of the study. Phosphate enhanced steaks performed better with respect to lean color than those enhanced with ammonium hydroxide. Fat color scores were also significantly different between treatments. The phosphate treatment had an average of 6.20 ± 1.19 and the ammonium hydroxide treatment 5.93 ± 1.30. The percent discoloration was significantly lower for phosphate than ammonium hydroxide enhanced steaks and overall acceptability was higher for phosphate enhanced steaks than ammonium hydroxide. For $L^*$ values, ammonium hydroxide treated samples were lighter than phosphate. Phosphate treated steaks were redder than ammonium hydroxide steaks based on $a^*$ value. There was not a difference in $b^*$, or yellowness, values between treatments.

Purge was 3.5% less for phosphate enhanced steaks than ammonium hydroxide. In addition, phosphate treated steaks loss less water than ammonium hydroxide treatments. There was not a significant difference for WBSF among days nor a day * treatment interaction. However, phosphate enhanced steaks were significantly more tender than ammonium hydroxide. Panelists found phosphate enhanced steaks more tender, juicier, and with less connective tissue than steaks enhanced with ammonium hydroxide. None of the categories analyzed for sensory panel were significantly different with regard to day or the say * treatment interaction except for connective tissue, which showed a significant difference only in the day * treatment interaction.

**Implications**
Enhancement of Select strip loin steaks with an ammonium hydroxide solution at pH 10 was not as effective as the industry-based phosphate injection solution. In addition, results suggest that there was a significant difference in performance between pH 10 and pH 11 ammonium hydroxide solutions. In general, the pH 10 solution did not sufficiently raise final meat pH. This affected final meat color stability, water holding ability and tenderness.

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