College of Agriculture & Life Sciences
Department of Animal Sciences
Meat Science and Muscle Biology Laboratory
1805 Linden Drive
Madison, WI 53706
Phone: 608-262-0875; Fax: 608-265-3110
E-mail: jrclaus@wisc.edu

Assessment of Potential Residues in Beef Associated with Application of Beef Carcass Vascular Rinse & Chill®

James R. Claus and Ko Eun Hwang
Meat Science and Muscle Biology Laboratory
Department of Animal Sciences
University of Wisconsin-Madison
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Introduction

The intent of this document is to provide a review of the historical validation testing as well as recent validation that assessed the potential for residues in beef associated with the food grade ingredients in the chilled isotonic rinse solution used to vascularly rinse out blood through the application of Rinse & Chill® technology. Immediately upon exsanguination, Rinse & Chill® (RC) is a process that entails inserting a sanitized catheter into the carotid artery and the rinse solution is then infused into the vasculature. Upon doing so, the rinse solution pushes the blood out of the carcass through the venous vasculature (jugular veins) and also continues to drain from the carcass similar to normal bleeding. The rinse solution is primarily composed of water (98.5%) with the balance being glucose, polyphosphates, and maltose. What is important to recognize is that glucose, polyphosphates, and maltose are used to enhance the glycolytic metabolism of the muscle. Phosphates stimulate energy metabolism and are naturally present in the muscle. Phosphatases also present in the muscle rapidly hydrolyze the phosphates as part of normal muscle metabolism. Glucose is the normal substrate in muscle used in muscle metabolism to produce energy. Maltose is simply a disaccharide composed of two glucose units which the muscle utilizes to provide additional glucose for metabolism. Since the rinse solution is introduced into the vasculature early postmortem, the muscle is physiologically fully active to utilize and consume these substrates.

Early Validation Associated with Residue Testing

The original residue study was conducted by staff from the University of Minnesota in 2000. There were 2 sampling periods – March 23 and 24, 2000 and April 17-19, 2000. The study was conducted on tissue collected from 216 cattle: 108 controls and 108 Rinsed cattle. There were 63 controls and 63 rinsed carcasses from the March timeframe and 45 controls and 45 Rinsed animals from the April period. All cattle were slaughtered at the same facility.

Sample preparation: Longissimus dorsi (LD) samples were collected from the fourth lumbar vertebra, 5 cm from the dorsal midline. Samples (~300 g each) were trimmed of all visible fat and placed into marked whirl pack bags and immediately placed into a cooler (3°C). Fifteen or 16 samples (Control and Rinsed) were collected each day, placed in a Styrofoam container and shipped to the University of Minnesota. A total of seven shipments were made. Composite

control or Rinsed samples were formed using equal weights of the lean material from each individual sample. The composite samples were ground through a 10 mm (3/8") plate and then twice through a 3 mm (1/8") plate following the AOAC moisture analysis protocol (AOAC 24.003-a). Samples were then taken from each composite and vacuum packed. Samples were then frozen and stored until further analysis.

Dextrose (glucose) analysis by HPLC

Frozen samples were thawed overnight at 3°C (38°F). A sample (20 g) of each LD composite was extracted with 40 ml 80 % ethanol/20% water in an Erlenmeyer flasks. Samples were then centrifuged at 2500 rpm for 10 min. Approximately 2 ml of the supernatant were micro filtered through 0.45 µm filters. Positive control samples (spiked samples) were also prepared by adding known amounts of dextrose to randomly chosen samples in order to check the percentage recovery rate. These spiked samples included beef muscle purchased from a local market as well as two of the composite groups (control and Rinsed). Duplicate injections for each composite were made for all of the 7 rinsed and 7 control composites.

The HPLC system was equipped with a Beckman Model 110A pump, a manual injector with 50 µL fixed sample volume, a Carbohydrate Analysis column (Waters Associates), a refractive index detector (Model R 401, Waters Associates) and a Chromato-integrator (Hitachi D-2000). The mobile phase was 80% acetonitrile/20% water and the separation was isocratic at a flow rate of 1 mL/minute. Samples were also concentrated 5x once it was determined that no detectable dextrose was found in any of the test samples.

Dextrose results: The results showed no dextrose peaks in any of the samples from the rinsed or unrinsed samples. Spiked samples had measurable dextrose in the positive controls. No differences between Rinsed and control samples were seen as all samples were below the detection limit of the procedure. In addition, no peaks were found in the samples that were concentrated 5x. These results indicate there was no measurable difference between rinsed beef and control beef.

Phosphorous Analysis

Frozen LD samples were thawed overnight at 3°C (38°F). Duplicate 1 g samples were weighed into ceramic crucibles and dried at 106°C for 6 hours. Samples were digested in HCL and then ashed in a 1500°C muffle furnace. The ash was then dissolved in ultra-pure HCL and subjected to analysis via an Inductively Coupled Plasma emission spectrograph (ICP). Standards and blanks were used at the beginning and end of the sample series to test the emission spectrograph for proper functioning. All phosphorus found in living tissue is in the form of phosphate. Therefore, phosphorus was analyzed and the results were expressed as both phosphorus and phosphate. A total of 14 samples were (7 rinsed and 7 controls) were analyzed.

Phosphorus results: Controls had an average of 2113 ppm phosphorus (6466 ppm expressed as PO_4 – phosphate). Rinsed product had 2079 ppm phosphorus (6362 ppm expressed as PO_4 – phosphate). Rinsed product only had a slight trend in having less phosphorus on the average than control product. In a very comprehensive study by Mateescu et al. (2013), they reported standard deviations of 249 µg/g (Iowa steer beef, n=309) and 278 µg/g (Iowa cow beef, n=231). Therefore, with the apparent difference between the control beef and the rinsed beef being only 34 µg/g, based on normal biological variability, one would conclude this was not a significant difference.

Conclusions from the University of Minnesota Study

The results demonstrate there was no differences in residual phosphate or dextrose between the rinsed beef and the control beef.

Recent Validation Testing of Residual Phosphorus and Sodium

A recent independent study was conducted to determine the effects of vascular rinsing and chilling temperatures on the quality attributes of meat from cull dairy cows. Carcasses from lean grade, cull dairy cows were conventionally chilled CN (n= 12) or RC processed (n = 28). Immediately after exsanguination at a commercial plant in North Carolina carcasses were vascularly rinsed (10% of the live body weight) with a chilled isotonic solution (RC3; Rinse & Chill® solution, 3°C; n = 13; RC14; Rinse & Chill® solution, 14°C; n = 15). Worth noting is that this slaughter plant did not have high voltage electrical stimulation that would also greatly accelerate postmortem metabolism and hence cause the muscle to more rapidly and potentially more completely consume available substrates. After the carcasses were chilled (24 h) carcass maturity was determined by evaluating the ossification of the bones and cartilage especially the split chine bones (sacral and lumbar vertebrae) by an experience grader. Carcasses from cull dairy cows 42 months or older were used for this study. After grading, muscles were excised and shipped overnight with freezer ice packs to the University of Wisconsin - Madison (averaged 3.1°C internal upon delivery). On day two postmortem the M. *Longissimus lumborum* (LL) were individually ground (19mm, 3mm plates) and vacuum packaged (vacuum setting, 10/10; model 2100-C; UltraVac-dual-chamber; Koch Equipment LLC., Kansas City, MO) in plastic pouches (2.7 mil thick, OTR 3-6cc/m2/24 h atm @ 4°C, 0 % relative humidity, blend of very-low-density polyethylene and ethylene vinyl acetate copolymer, 18 x 30 cm, product code 9KN81, Sealed Air Corporation). Packaged samples were stored (3°C) prior to being placed in a freezer (-25°C) on day 2 postmortem.

Ground samples (~150 g each) were shipped (11/4/2019) frozen overnight to an independent certified lab (Deibel Laboratories, Lincolnwood, IL) for residue testing. This lab analyzed the samples for total phosphorus and total sodium using modern inductively coupled plasma methodology (ICP, AOAC 985.01). The data were analyzed using the SAS MIXED procedure (SAS 9.1.3 Service Pack 3, SAS Institute Inc., Cary, NC, USA) to determine significance ($P < 0.05$) in the model. When significance was found, letter assignment to individual means to enable statistical comparisons was achieved using the pdmix800 macro (Saxton, 1998).

As documented in the early residue validation work in which meat from RC carcasses that were vascularly rinsed with the cold solution (3°C) the concentration of residual phosphorus was not different than the non-rinsed control samples (Figure 1). In addition, that beef from carcasses rinsed at the higher rinse solution temperature was also not different than the control. These values are similar to those reported by Mateescu et al. (2013) for conventionally chilled carcasses. They analyzed the phosphorus content of the longissimus muscle and reported 1742 µg/g in Iowa Angus cow beef and 1759 µg/g in Iowa Angus steer beef.

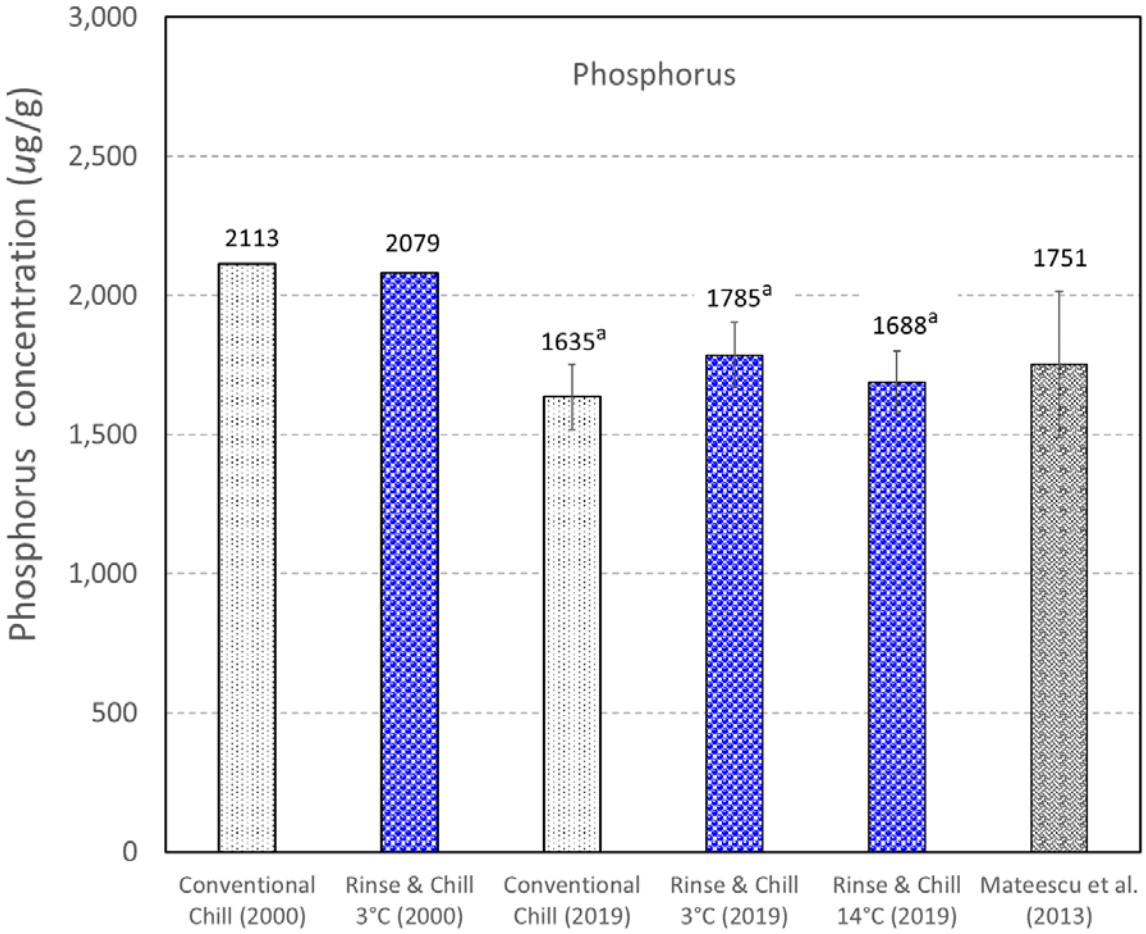

Figure 1. Phosphorus content (mean ± std. dev.) of beef longissimus muscle from conventionally chilled carcasses compared to Rinse & Chilled® carcasses from two independent studies (2000, 2019). [a]Means from the 2019 study demonstrated no differences (P>0.05, S.E.=115.3) were found in the beef samples between the non-rinsed controls and either of the different rinse solution temperatures. The Mateescu et al. (2013) results represent the average of steer and cow values from conventionally chilled beef.

The phosphate used to make up the rinse solution would be in the form of a sodium salt of the phosphate. The sodium content in the RC ground longissimus muscle from beef carcasses rinsed with either solution temperature also was not different than the control samples (Figure 2).

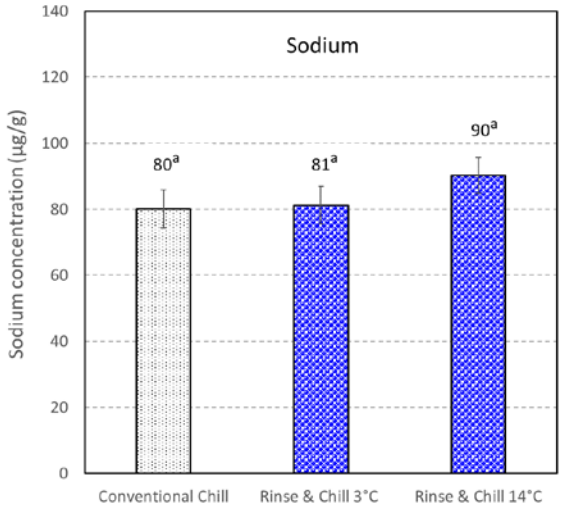

Figure 2. Sodium content (mean ± std. dev.) of beef longissimus muscle from conventionally chilled carcasses compared to Rinse & Chilled® carcasses (2019 study). [a]Means with the same superscript letter are not different (P>0.05. S.E.= 5.62).

Conclusions

Prerigor muscle is physiologically active and therefore capable of metabolizing endogenous as well as added sources of glucose. In addition, endogenous phosphate is also involved in normal muscle metabolism. As such, the minor amount of these substances used to rinse out the blood from the vasculature, even if none of them drained from the carcass, would be readily metabolized by the muscle. Based on the early validation work performed by the University of Minnesota combined with the recent validation testing, supports the conclusion there is no difference in residuals between conventionally chilled carcasses and carcasses that are vascularly rinsed using the Rinse & Chill® process.

References

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