Lactate Dehydrogenase Activity in Bovine Muscle as a Potential Heating Endpoint Indicator

Sharen S. Collins, Jimmy T. Keeton,* and Stephen B. Smith

Meats and Muscle Biology Section, Department of Animal Science, Texas A&M University, and Texas Agricultural Experiment Station, College Station, Texas 77843

Lactate dehydrogenase (LDH) activity was measured in heated extracts of cooked bovine muscle tissues to evaluate the potential for developing a rapid, accurate assay to verify cooking endpoint temperatures in beef. Adductor and semimembranosus muscles from six Angus steers were assigned to one of four treatments: fresh, frozen, frozen–thawed, or aged. Each treatment was applied to beef rounds that did or did not receive electrical stimulation. LDH activity rates were monitored in heated and unheated samples following 3 weeks of vacuum-packaged storage at 4 °C. Treatments other than heating showed little effect on LDH activity. A major portion of activity was lost upon heating to 63 °C, and only marginal activity was detectable at 66 °C. Extractable protein decreased with increasing temperature. Decreases in LDH activity with increasing temperature may result from heat denaturation of the enzyme and/or from decreased amounts of extractable protein.

INTRODUCTION

Food-borne diseases are a constant and potentially serious threat to human health. The economic impact of food-borne diseases in the United States in 1988 has been reported to exceed $8.4 billion (Todd, 1989). Although Salmonellae frequently infect foodstuffs and sometimes result in salmonellosis, it is difficult to estimate their contribution to the total economic loss. In 1979, 6 of 10 of the most frequently reported Salmonellae serotypes from humans in the United States also ranked among the 10 most frequently found in nonhuman sources (Schwabe, 1984). Poultry, beef, and pork are common vectors of salmonellosis in humans. Of the outbreaks of salmonellosis reported in the United States in 1979 that could be traced to an identifiable source, 40% of the infections resulted from the consumption of red meat (Schwabe, 1984).

Salmonellae are very heat sensitive, but the presence of these bacteria in food results from inadequate heat treatment or more likely postheating contamination (Silliker, 1986). Domestic cooked beef and roast beef products are required by the USDA Food Safety and Inspection Service (FSIS) to be cooked to a minimum internal temperature of 54.5 °C (131 °F) for 121 min or to 62.8 °C (145 °F) (USDA-FSIS, 1982). This treatment destroys Salmonellae and renders the beef safe for human consumption. A protein coagulation test currently is employed by the FSIS to determine the maximum internal temperature to which beef products have been cooked. This procedure, however, is empirical and highly subjective, clearly establishing a need for more reliable and accurate thermal testing procedures (Townsend et al., 1984).

From a companion study (Collins et al., 1991), lactate dehydrogenase (LDH) activity in uncured porcine muscle was found to decrease upon heating to a maximum internal temperature of 65–68 °C. In cured pork muscle, virtually all LDH enzyme activity was lost when heated to 63.8 ± 0.4 °C. The combination of curing and heating appeared to accelerate the loss of LDH enzyme activity, while heating alone resulted in a progressive loss of enzyme activity with increasing temperature. Cooked beef and roast beef products typically are not cured, unlike hams, and are heated to a lower minimum internal temperature, 62.8 °C. Because of the similarity between temperature endpoints observed for loss of LDH activity and the minimum endpoint required for roast beef products, the potential exists for development of a temperature endpoint verification test based on LDH activity of cooked beef products.

The objective of this study was to analyze LDH activity in beef top round muscles that received processing treatments similar to those found in the meat industry. If loss of LDH activity occurs at a temperature that is consistent with the USDA-FSIS minimum internal temperature requirement for cooked beef products, then an accurate temperature verification test potentially could be developed.

MATERIALS AND METHODS

Muscles of the top round (semimembranosus and adductor) were obtained from six Angus steer carcasses at 16 months of age, ranging in weight from 328.9 to 419.5 kg. Top rounds were removed from the carcasses following a 24-h chilling period at 4 °C and assigned to one of the following treatments: fresh; aged; electrically stimulated (ES); frozen–thawed; aged–ES; aged–frozen–thawed; ES–aged–frozen–thawed; ES–frozen–thawed. Table I reports the treatment assignments for the top rounds used in this study.

Muscles designated as aged were stored at 4 °C for 7 days after being double-wrapped in waxed freezer paper. Frozen samples were double-wrapped and held at −24.5 ± 0.4 °C for 5 days followed by a thawing period of 2–3 days at 4 °C and 1 day at 25 °C. The internal meat temperature after thawing was 5.9 ± 2.7 °C when tested and was monitored by using an Omega 871 digital thermometer connected to a chromel/alumel insulated thermocouple inserted into the geometric center of each top round. Electrically stimulated rounds were taken from individual carcass sides which had been stimulated by using a Koch-Britton probe inserted into the neck region of the carcass. Stimulation was induced at 200–300 V, 3 A, 60 Hz, and 19 pulses per minute. Tissue pH was determined on each round after the appropriate treatment was given by inserting a probe-type pH electrode into the muscle mass and reading the pH from an Orion digital ionalyzer (Model 601A).

Stainless steel tubes, 15.24 cm long with a 1.3 cm outside bevel on one end (cutting edge), an inner diameter of 3.6 cm, and an outer wall thickness of 3 mm, were used to core the raw muscle

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Table I. Treatment Assignment for Each Top Round Used in This Study

animal	treatment round 1	treatment round 2
1	fresh[a]	ES[b]
2	fresh	AG[c]
3	FZ-TH[d]	ES-FZ-TH
4	FZ-TH	AG-FZ-TH
5	AG	ES-AG
6	AG-FZ-TH	ES-AG-FZ-TH

[a] Fresh, no additional treatment was applied to the round once it was obtained from the carcass following a 24-h chill at 4 °C. [b] ES, the carcass was electrically stimulated by using 200–300 V, 3 A, 60 Hz, and 19 pulses/min. The rounds were obtained from the carcasses following a 24-h chill at 4 °C. [c] AG, the rounds were aged 7 days at 4 °C after they were removed from the carcasses following a 24-h chill at 4 °C. [d] FZ/TH, following a 24-h chill at 4 °C, the rounds were removed from the carcasses. The rounds were then frozen at −24.5 ± 0.4 °C for 5 days followed by thawing at 4 °C for 2–3 days and 1 day at 25 °C.

Table II. Analysis of Variance Table

	source DF	type III SS	F	PR > F
Tests of Hypothesis Using the Type III MS for Round × Treatment as an Error Term				
treatment	7	387 104.30	3.08	0.1470
Tests of Hypothesis Using the Type III MS for Round × Treatment × Temperature as an Error Term				
temperature	4	12 212 545.38	73.54	0.0001

samples. The tube ends were sealed with rubber stoppers, and a 14-gauge needle was inserted into one end, allowing for the passage of a 30-gauge chromel/alumel thermocouple wire into the geometric center of the meat core. The meat samples were heated in a constant temperature water bath to 54.5 ± 0.4 °C and held for 121 min or to 60.0, 62.8, or 65.6 °C without holding. The water bath temperature was held at 4 °C above the endpoint internal temperature of the meat. After reaching the specified temperature for the proper time period, the samples were placed in Whirl Pak bags and submerged in ice water to halt the heating process. The heated and unheated samples were vacuum-packaged, stored at 4 °C for 3 weeks, and then analyzed for enzyme activity.

Homogenate sample preparation consisted of homogenizing 2 g of tissue with 8 mL of 0.01 M phosphate buffer in a Virtis 45 homogenizer (for 30 s at speed 40). The homogenate was centrifuged (Beckman J2-21, 13823g, 30 min, 4 °C), the fat was aspirated from the enzyme suspension, and 2-mL aliquots of supernate were diluted with 0.01 M phosphate buffer to yield a 200:1 sample dilution.

The LDH activity of each sample was determined by using a Beckman DU-7 spectrophotometer according to the procedure of Vassault (1983). Two samples were taken from each muscle within a treatment combination, and enzyme assays were performed in duplicate. Thus, the reported enzyme activities are an average of four enzyme reactions per sample. Extractable protein was measured according to the procedure of Lowry et al. (1951).

Analysis of variance was conducted by using the general linear model procedure of SAS (1986). LDH activities were analyzed by using a split-plot design blocking by round, with treatment and the treatment × temperature interaction in the whole plot. Temperature, treatment × temperature, and round × treatment × temperature were in the split plot. When significant differences in treatment effects were noted, mean activity rates were compared by using Tukey's Studentized range test.

RESULTS AND DISCUSSION

The analysis of variance table (Table II) illustrates that the different treatments applied to the top rounds did not significantly affect the LDH activity rates. However, the temperature to which the muscle tissues were heated did have a significant effect on enzyme activity. Thus, the LDH activity rates were pooled across all treatments and are reported as mean activity rates (n = 48) in Table III. LDH activity in raw muscle tissue, 649.23 ± 14.00 μmol/(min·g), significantly decreased upon heating to 55 °C, a 26.6% reduction. When the tissue was heated to 60 °C, a 33.7% reduction in LDH activity occurred when compared to the raw tissue. Likewise, when raw muscles were heated to 63 and 66 °C, significant reductions of 77.1 and 96.5% in LDH activity resulted, respectively.

Table III. Mean Lactate Dehydrogenase Activity as a Function of Temperature[a]

temp, °C	n	lactate dehydrogenase activity,[b] μmol/(min·g)
4	48	649.23 ± 14.00[a]
55	48	476.52 ± 10.52[b]
60	48	430.76 ± 14.20[c]
63	48	148.44 ± 16.34[d]
66	48	22.42 ± 3.64[e]

[a] Mean lactate dehydrogenase activities are an average of the pooled treatments. [b] Mean lactate dehydrogenase activity ± standard error of the mean. Means with common superscripts are not significantly different (p > 0.05).

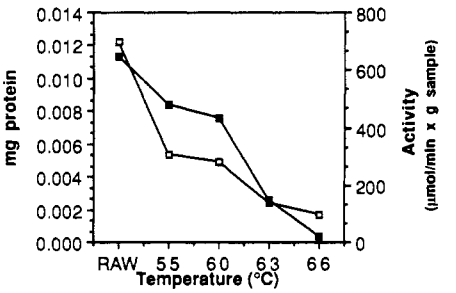

Figure 1. Average lactate dehydrogenase activity (■) and average milligrams of extractable protein (□) in bovine top round muscles as a function of temperature.

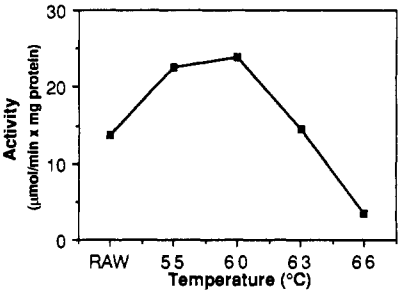

Figure 2. Average lactate dehydrogenase specific activity rates in bovine top round muscles as a function of temperature.

To explain the differences in activity observed with increased heating, extractable protein in the diluent was determined for each temperature/treatment combination (Figure 1). As the samples were heated to 55, 60, 63, and 66 °C, the amount of total extractable protein decreased by 56.2, 59.6, 80.4, and 85.8%, respectively, when compared to that of unheated samples. Average LDH activities, measured across all treatment combinations, exhibited an inverse relationship with temperature. As the temperature increased to 55, 60, 63, and 66 °C, overall LDH activity decreased by 26.4, 33.6, 77.1, and 96.5%, respectively, when compared to that of the unheated samples.

The data in Figure 1 suggest that the reduction in LDH activity with cooking is the result of reduced extractable protein in the supernates. It is also possible that heating reduced the specific activity of LDH. Specific activities, averaged across all treatments, are shown in Figure 2. Unheated samples had the highest activity and largest

amounts of extractable protein. Thus, the specific activity [13.79 μmol/(min·mg)] was lower than that of muscles heated to 55 and 60 °C [22.62 and 23.88 μmol/(min·mg), respectively]. At these temperatures, activity rates remained relatively high, and the amount of extractable protein dropped precipitously with increasing temperature (Figure 1). Therefore, the ratios of activity to milligrams of extracted protein were higher than that observed in the unheated samples. Heating to 63 °C resulted in a large drop in LDH activity as well as a large decline in the amount of extractable protein. However, the resulting specific activity ratio [14.52 μmol/(min·mg)] was similar to the activity observed for the unheated samples. Although activity rates for unheated samples and those heated to 63 °C were very similar, the unheated samples had high LDH activity accompanied by large amounts of extractable protein. The 63 °C samples, conversely, showed low activity rates coupled with small amounts of extractable protein. The low specific activity at 66 °C [3.52 μmol/(min·g)] indicated that pronounced enzyme denaturation had occurred at this cooking temperature or that a large loss of protein occurred. Thus, the marked decline in LDH activity observed at 66 °C (Table III) resulted from the combination of reduced extractable protein and denaturation of LDH. At lower temperatures, extraction of LDH appeared to be the primary determinant of LDH activity.

Cooked and roast beef products are required to be processed to a minimum internal temperature of 54.5 °C for 121 min or 62.8 °C with no holding time. At the latter endpoint temperature, all treatments exhibited a large drop in LDH activity. Because of the temperature sensitivity range indicated by LDH activity, there appears to be great potential for developing an accurate maximum internal temperature test for cooked and roast beef products based on LDH activity.

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