Lactate Dehydrogenase Enzyme Activity in Raw, Cured, and Heated Porcine Muscle

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Semimembranosus, semitendinosus, biceps femoris, rectus femoris, and adductor muscles were dissected from nine fresh hams. Each muscle was analyzed for lactate dehydrogenase (LDH) activity after receiving one of the following treatments: fresh, aged, frozen, frozen–thawed, heated, cured, and cured–heated. Aging resulted in an increase in LDH activity, while freezing and freezing–thawing resulted in a marked decrease in activity. Heating fresh tissue to 65–68 °C resulted in large activity losses. Heating to 68 °C and above significantly inactivated the enzyme. Curing and heating to 63.8 °C eliminated almost all LDH activity. Pyruvate kinase, malate dehydrogenase, adenylate kinase, isocitrate dehydrogenase, creatine kinase, aspartate aminotransferase, fructose-1,6-bisphosphate aldolase, citrate synthase, and glutamate-oxaloacetate transminase activities were monitored in cured ham muscle heated to 64.5 and 68.8 °C. No appreciable enzyme activity was detected in any cured/heated samples.

INTRODUCTION

Communicable swine diseases such as African swine fever, foot and mouth disease, hog cholera, and swine vesicular disease are prevalent in many countries worldwide. Importation of underprocessed pork products originating in countries laden with such diseases could lead to the introduction of these diseases into the United States. Thus, protective measures are employed by the USDA to ensure the importation of disease-free meat. In 1986, pork imported into the United States equaled 7.9% of the U.S. domestic production (American Meat Institute, 1987). However, of the total amount of canned, pasteurized hams and pork shoulders, 311.4 million pounds were imported while only 95.1 million pounds were canned under U.S. federal inspection.

The causative viruses of communicable swine diseases are thermolabile. Therefore, the USDA Animal and Plant Health Inspection Service/Veterinary Services (APHIS/VS) requires imported canned pork products to be cooked to an internal temperature of 68.9 °C (USDA-FSIS, 1986). A heat treatment greater than or equal to 68.9 °C destroys the viruses and renders the meat products safe for human consumption.

An acid phosphatase test is currently being used to verify the maximum internal temperature to which imported canned pork products have been heated. Evaluations of this procedure, however, have shown residual acid phosphatase activity is an inaccurate test for determining the actual thermal processing endpoint (Cohen, 1969). The development of a test based on activities of other enzymes within the muscle tissue could provide meat processors and the USDA with a dependable and accurate means of monitoring the maximum internal temperature achieved during thermal processing. This would enhance quality control procedures and, in turn, ensure a wholesome product for the consumer.

Therefore, the objective of this study was to identify a porcine muscle tissue enzyme that loses a large portion of activity after curing and heating to 68.9 °C. On the basis of this information, a maximum internal temperature verification test for cured, canned pork products could be developed.

MATERIALS AND METHODS

Nine fresh hams, weighing 6.3–7.7 kg, were obtained 3 days postslaughter (ps) from typical martket weight carcasses held at 4 °C. The semimembranosus (SM), semitendinosus (ST), biceps femoris (BF), and rectus femoris (RF) muscles of the nine hams were dissected and closely trimmed of all connective tissue membranes and seam (intermuscular) fat. The ham muscles were treated as follows:

Ham 1, Fresh. The four muscles were stored individually in Whirl Pak bags for 2 days at 4 °C. On day 5 ps, 4–6-g samples from each muscle were analyzed for LDH activity.

Ham 2, Frozen. Dissected muscles were individually double-wrapped in freezer paper and held at −10 °C for 8 days. On day 11 ps, 5–7 g of each muscle was analyzed for LDH activity.

Ham 2, Frozen and Thawed. After frozen muscle samples were obtained from ham 2, the remaining muscles were wrapped singly in freezer paper and allowed to thaw for 24 h at 4 °C. Samples (5–7 g) of each muscle were analyzed for LDH activity.

Ham 3, Aged. The ham was stored at 4 °C for 3 days, and individual muscles were dissected, placed in Whirl Pak bags, and held for an additional 7 days at 4 °C. On day 11 ps, 3–5-g samples of each muscle were analyzed for LDH activity.

Hams 4–6, Heat-Treated. Nine 11–15-g whole muscle samples were taken from each of the four muscles of each ham, placed in polycarbonate tubes, and heated to specified endpoint temperatures in a constant-temperature water bath. The water bath temperature was maintained at 4 °C higher than the desired endpoint internal temperature of the meat sample. Samples from each muscle received one of nine heating time/temperature treatments as follows: 65 °C for 8, 23, or 38 min; 69 °C for 8, 23, or 38 min; 71 °C for 8, 23, or 38 min.

Internal temperatures were monitored by using a 30-gauge copper/constantan insulated thermocouple wire positioned in the geometric center of each sample and connected to a Honeywell recorder. After the specified temperature was reached and held for the proper time period, the samples were drained of all juices, placed in Whirl Pak bags, and submerged in ice water to halt the heating process. Core samples of 3–4 g, obtained from the geometric center of each sample, were retained for LDH activity analysis.

Hams 7–9, Heat-Treated and Cured. The SM and adductor (AD) muscles were designated group A, while BF and ST were designated group B, resulting in six test groups: 1A, 2A, 3A, 1B, 2B, and 3B. Each muscle group was bisected, allowing for half of the samples to be tested uncured and the opposing section to be tested following cure application.

By use of a needle and syringe, muscle samples designated as cured were injected with brine formulated to contain 15% sodium

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chloride, 1.25% sucrose, 0.50% sodium tripolyphosphate, 0.42% sodium erythorbate, and 0.10% sodium nitrite.

Following cure injection at 15% of the fresh muscle weight, the samples were held in a brine soak at 5 °C for 65 h. The brine soak consisted of the same mixture of ingredients as the injected brine minus sodium erythorbate. Muscle samples, weighing 11–15 g, were cored and extracted by using a 1.5-cm cork borer. Uncured samples were placed in polycarbonate tubes filled with double-distilled water and heated in a constant-temperature water bath. The water bath temperature was maintained 4 °C above the endpoint internal temperature of the muscle samples. The cured samples were placed in tubes containing the brine soak and heated to the same respective temperatures.

Each muscle group was subjected to four heating time/temperature treatments as follows: 64 °C for 8 or 23 min; 68 °C for 8 or 23 min.

The temperature of each sample was maintained at the desired endpoint for the appropriate holding time and monitored with an Omega 871 digital thermometer connected to 30-gauge chromel/alumel insulated thermocouples positioned in the geometric center of each muscle sample. After the specified time/temperature endpoint was reached, heated samples were placed in Whirl Pak bags and immediately submerged in ice water to halt the heating process. Samples (1–2.5 g) were taken from the geometric center of each heated sample and prepared for enzyme activity analysis.

Homogenate Preparation. Following the prescribed treatment, 1–2.5-g samples of muscle were homogenized in a Virtis 45 homogenizer (speed 40, 30 s) with 8 mL of 0.01 M potassium phosphate buffer solution (pH 7.2). The homogenate was centrifuged at 13823*g* for 30 min at 4 °C. The supernate fat cake was aspirated, and an aliquot of infranate enzyme suspension was diluted with 0.01 M potassium phosphate buffer (pH 7.2) to either a 320:1, 360:1, or 400:1 dilution.

By use of a Beckman DU-7 spectrophotometer, LDH activity of each sample was determined according to the procedure of Vassault (1983). One unit (U) of activity is defined as 1 μmol of pyruvate reduced to lactate per minute per gram of protein. Aspartate aminotransferase (AAT) activity was determined according to the procedure of Rej and Horder (1983). Analysis procedures of Gehardt (1983) and Willnow (1983) were followed to determine activities of creatine kinase (CK) and fructose-1,6-bisphosphate aldolase (ALD), respectively. Bergmeyer and Bernt (1983), Goldberg and Ellis (1983), and Smith (1983) procedures were used to determine glutamate-oxaloacetate transaminase (GOT), isocitrate dehydrogenase (ICDH), and malate dehydrogenase (MDH) activities, respectively. Pyruvate kinase (PK) activity was determined according to the procedure of Fujii and Miwa (1983). Adenylate kinase (AK) was determined according to the procedure of Brolin (1983), and citrate synthase (CS) activity was monitored as described by Stitt (1983).

Analysis of variance was conducted by using the general linear model procedure of SAS (1986). LDH activities in hams 7–9 were analyzed by using a split-plot design blocking by hams. When significant treatment effects were noted, mean activity rates were compared by using Tukey's Studentized range test.

RESULTS AND DISCUSSION

Muscles vary in their content and distribution of red and white fiber types. White fibers contain primarily the M4 isozyme of LDH, while the H4 isozyme predominates in red fibers. Pyruvate and NADH are substrates for LDH in the reaction pyruvate + NADH + $H^+ \overset{LDH}{\rightleftarrows}$ L-(+)-lactate + NAD^+. The LDH isozymes differ in their effectiveness of NADH utilization; depending on which fiber type is dominant in individual muscles, different LDH activity rates result (Table I). Semimembranosus (SM) and rectus femoris (RF) porcine muscles are predominantly composed of red fibers, while semitendinosus (ST) and biceps femoris (BF) muscles contain primarily white fibers. In this study, fresh BF muscle tissue was found to exhibit lower LDH activities than SM, RF, and ST. However, no comparisons of LDH activity between fiber types were made in this study because ST and BF both have areas where either red or white fibers predominate and sampling was done randomly.

Table I. Average Lactate Dehydrogenase Activity in Fresh, Aged, Frozen, and Frozen and Thawed Ham Muscles[a]

muscle	*n*	lactate dehydrogenase act., μmol/(min·g)
fresh[b]		
biceps femoris	10	460.67 ± 25.78[a]
rectus femoris	10	757.53 ± 61.72[b]
semimembranosus	10	743.95 ± 25.62[b]
semitendinosus	10	653.27 ± 32.59[b]
aged[c]		
biceps femoris	10	1187.12 ± 15.70[c]
rectus femoris	10	949.86 ± 17.59[d]
semimembranosus	10	802.52 ± 27.94[b]
semitendinosus	10	493.91 ± 18.05[a]
frozen[d]		
biceps femoris	10	120.55 ± 39.78[g]
rectus femoris	10	337.79 ± 23.14[f]
semimembranosus	10	501.38 ± 31.26[a]
semitendinosus	10	113.41 ± 35.57[e]
frozen and thawed[e]		
biceps femoris	10	64.76 ± 11.14[e]
rectus femoris	10	101.53 ± 25.76[e]
semimembranosus	10	191.72 ± 76.52[e]
semitendinosus	10	20.82 ± 7.93[e]

[a] Lactate dehydrogenase activity is reported as the mean ± standard error of 10 reactions. Mean values with common superscripts are not significantly different ($p > 0.05$). [b] Fresh muscles were dissected from hams and stored for 2 days at 4 °C in Whirl Pak bags. [c] Aged muscles were dissected from hams following a 3-day storage at 4 °C, placed in Whirl Pak bags, and held for an additional 7 days at 4 °C. [d] Frozen muscles were dissected from hams, double-wrapped in freezer paper, and held at −10 °C for 8 days. [e] Frozen and thawed muscles were obtained from the frozen samples, wrapped singly in freezer paper, and allowed to thaw for 24 h at 4 °C.

During post-mortem aging of muscle, enzymatic degradation of the muscle structure occurs. Lysosomal enzymes partially degrade myosin and actin, but most of the connective tissue and myofibrillar proteins are not extensively proteolyzed. The observed increase in soluble products resulting from protein hydrolysis during aging must come from the breakdown of sarcoplasmic proteins (Lawrie, 1985). Thus, the activity of LDH, a sarcoplasmic protein, may be affected during aging of the muscle.

LDH activity was significantly higher in aged BF and RF muscles when compared to fresh muscle LDH values (Table I). LDH activity in aged SM was not significantly different from the activity in fresh SM, 802.52 ± 27.94 and 743.95 ± 25.62 U, respectively. LDH activity in aged ST was significantly lower than in fresh ST, 493.91 ± 18.05 and 653.27 ± 32.59 U, respectively. Freezing caused significant reductions in LDH activity in each of the four muscles. Freezing reduced LDH activities by 73.8, 55.4, 32.6, and 82.6% in BF, RF, SM, and ST muscles, respectively, when compared to that in fresh muscle tissue (Table I). The LDH-4 and LDH-5 isozymes of lactate dehydrogenase are cold labile and have been shown to lose up to 20% of their activity within 3 days at 4 °C (Lott and Stang, 1980). Loss of activity in these isozymes is a probable cause for the significant decline in LDH activity in the frozen samples. Another contributing factor to the loss of enzyme activity may be protein loss from the frozen tissues. Upon freezing, ice crystals form within the muscle tissues and puncture cell membranes, allowing water-soluble nutrients such as amino acids, salts, proteins, and peptides to be lost as drip (Forrest et al., 1975). Freezing and thawing resulted in even greater losses of enzyme

Table II. Average Lactate Dehydrogenase Activity of Ham Muscles Heated to Various Endpoint Temperatures as a Function of Holding Time[a]

	holding time		
temp, °C	8 min	23 min	38 min
65	274.88 ± 15.33[a]	275.54 ± 31.56[a]	438.83 ± 17.67[c]
68	91.63 ± 21.85[b]	78.37 ± 15.46[b]	53.87 ± 8.74[b]
71	40.35 ± 9.27[b]	18.17 ± 5.06[b]	30.27 ± 10.03[b]

[a] Lactate dehydrogenase activity [μmol/(min·g)] is reported as the mean ± standard error (n = 20) pooled across muscles. Means in a row or column with common superscripts are not significantly different ($p > 0.05$).

Table III. Average Lactate Dehydrogenase Activity in Uncured and Cured Ham Muscles as a Function of Different Time/Temperature Combinations[a]

treatment	temp, °C	holding time, min	lactate dehydrogenase act., μmol/(min·g)
uncured	4.0	1	734.80 ± 57.73[a]
cured	4.0	1	764.95 ± 75.22[a]
uncured	63.8	8	423.11 ± 41.95[b]
cured	63.8	8	116.06 ± 34.84[c]
uncured	63.8	23	104.92 ± 34.34[d]
cured	63.8	23	23.95 ± 11.96[e]
uncured	68.8	8	36.34 ± 16.32[f]
cured	68.8	8	3.26 ± 0.66[f]
uncured	68.8	23	0.68 ± 0.19[g]
cured	68.8	23	4.20 ± 1.06[b]

[a] Lactate dehydrogenase activities are the mean ± standard errors pooled across muscles (n = 6). Means within a time/temperature combination with common superscripts are not significantly different ($p > 0.05$).

activity than did freezing (Table I). When compared to freezing alone, freezing–thawing resulted in a decline in LDH activity of 46.3, 69.9, 61.8, and 81.6% in BF, RF, SM, and ST, respectively.

Table II reports an overall decline in LDH activity of the muscles of three hams (hams 4–6) heated to 65.0, 68.0, and 71.0 °C and held at each endpoint temperature for 8, 23, and 38 min. Temperature appears to be the major factor affecting LDH activity, rather than the time held at various temperatures. Heating porcine muscles to temperatures greater than or equal to 68 °C resulted in virtually no LDH activity in any of the samples, regardless of holding time. Between 65 and 68 °C, the porcine muscle samples lost a significant amount of LDH activity. The mean activity over all holding times was 329.75 U when heated to 65 °C, 74.62 U when heated to 68 °C, and 29.60 U when heated to 71 °C. The 38-min holding time resulted in the highest activity at 65 °C, 438.83 U, but when heated to 68 and 71 °C, the LDH activity in muscles held for 38 min was not significantly different from those held for 8 and 23 min.

LDH activities in unheated, uncured muscle halves were not different from the activities in the opposing muscle halves that were unheated and cured (Table III). Upon heating to 63.8 ± 0.4 °C for 8 min, however, LDH activity in the uncured samples was 72.6% higher than in the cured muscle counterpart. However, heating to 63.8 ± 0.4 °C resulted in significant LDH activity reductions in both uncured and cured samples, 42.2 and 84.8%, respectively. Increasing the temperature to 68.8 ± 0.8 °C resulted in virtually no LDH activity in either the uncured or cured samples at all holding times.

A 23-min holding period at 63.8 ± 0.4 °C reduced LDH activity in uncured muscle when compared to the 8-min holding period, 104.92 ± 34.34 and 423.11 ± 41.95 U, respectively (Table III). When compared to the unheated, uncured samples, LDH activities dropped 42.4% in the samples held for 8 min and 85.7% in samples held for 23 min.

Cured samples heated to 63.8 ± 0.4 °C and held for 8 min showed an 84.8% decrease in activity when compared to the unheated, cured samples (Table III). Likewise, LDH activity of cured samples heated to 63.8 ± 0.4 °C and held for 23 min approached zero. The combined effects of curing and heating virtually eliminated LDH activity at an approximate endpoint temperature of 63.8 °C, regardless of the holding time. Because of the loss of LDH activity in ham muscles heated to temperatures well below 68.9 °C, this enzyme does not appear to be useful in monitoring the processing endpoint temperatures of cured, canned hams.

Table IV. Mean Activity Rates of Nine Enzymes in Cured and Heated Porcine Ham Muscle[a]

	μmol/(min·g of tissue) at endpoint temp of	
enzyme	64.5 °C	68.8 °C
adenylate kinase	−3.33 ± 0.36	−5.12 ± 0.090
aldolase	−2.58 ± 0.80	−2.72 ± 1.38
aspartate aminotransferase	−5.05 ± 0.14	−10.38 ± 0.55
citrate synthase	2.31 ± 0.78	1.86 ± 1.47
creatine kinase	1.41 ± 0.14	7.04 ± 0.32
glutamate-oxaloacetate transaminase	−0.65 ± 0.0076	−0.72 ± 0.034
isocitrate dehydrogenase	−0.65 ± 0.31	−0.71 ± 0.35
malate dehydrogenase	0.0060 ± 0.010	0.0045 ± 0.0076
pyruvate kinase	−2.53 ± 0.63	−0.57 ± 0.21

[a] Means ± standard errors are averages of four determinations.

Activities of nine other enzymes found in high concentration in muscle tissue were evaluated (Table IV). Activities of these enzymes were analyzed in porcine muscle tissue that was cured and heated, according to the same procedures used for the assay of LDH activity. As was observed with LDH, the activity of each enzyme was virtually undetectable in the cured muscle heated to 64.5 and 68.8 °C.

Due to the significant loss of activity when heated to temperatures well below 68.9 °C, none of the 10 enzymes in cured and heated porcine muscle evaluated in this study exhibited properties which would indicate their potential for use as an indicator of endpoint heating temperatures in canned, cured hams. However, further enzyme activity studies using porcine muscle subjected to other commercial processing conditions may yield more favorable results.

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