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# LACTATE DEHYDROGENASE ACTIVITY IN PORCINE MUSCLE AS INFLUENCED BY FREEZING, THAWING, AGING, CURING AND HEATING

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SUMMARY

Aging of four porcine muscles from the ham caused an increase in LDH activity rates in two groups when compared to fresh muscles. Freezing and freezing/thawing muscles depressed LDH activity rates in all muscles in comparison to fresh tissue. Heating uncured ham muscle to temperatures ranging from 62.8°C to 64.8°C resulted in a large loss of LDH activity. Increasing temperatures to 68.0°C and above resulted in virtually no observable LDH activity. Curing alone did not have a marked effect on the LDH activity in raw ham tissue samples. However, the combination of curing and heating resulted in a large decrease in LDH activity when ham muscles were heated to temperatures ranging from 63.6 to 67.2°C. This temperature range falls below the minimum temperature required for imported canned pork products, 68.9°C. Therefore, LDH does not appear to be a useful enzyme indicator for determining the maximum internal temperature achieved in processing these meat products.

## INTRODUCTION

The total pounds of pork imported into the U.S. in 1984 equaled 6.4% of the U.S. domestic production (American Meat Institute, 1985). In that same year, 296.5 million pounds of canned hams and shoulders were imported into the U.S., whereas only 155.2 million pounds were canned domestically under U.S. federal inspection. Because destructive communicable swine diseases such as African swine fever, foot and mouth disease, hog cholera and swine vesicular disease infect many countries worldwide importation of underprocessed pork products originating from infested countries could lead to the introduction of these diseases into the United States.

Protective measures are employed by the USDA to ensure the importation of disease-free meat. The causative viruses of communicable swine disease are thermolabile, therefore, the USDA Animal Plant Health Inspection Service/ Veterinary Services (APHIS/VS) requires that imported canned pork products be cooked to an internal temperature of 69°C (USDA-FSIS, 1986). By heating to 69°C, the viruses are destroyed rendering the meat products safe for human consumption.

The acid phosphatase test (Lind, 1965) is currently being used to verify the maximum internal temperature to which imported canned pork products have been heated. Variability in some test results from this procedure have lead to a re-evaluation of the residual acid phosphatase activity test as an accurate measure of thermal processing (Cohen, 1969). The development of a maximum internal temperature test based on activity rates of other enzymes within the muscle tissue could provide meat processors and the USDA with a monitoring tool that would be easy to use and produce rapid, accurate results. 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 and quantify a porcine muscle enzyme that loses a proportionate amount of activity when heated to 69°C. Based on this information, a maximum internal temperature verification test for cured, canned pork products could be developed. After an initial screening of eleven sarcoplasmic enzymes lactate dehydrogenase (L-lactate: NAD, oxidoreductase, EC 1. 1. 1. 27) LDH was selected for further testing because of its relative abundance and sensitivity to heating near 69°C.

## MATERIALS AND METHODS

Nine fresh hams, ranging in weight from 6.3 to 7.7 kg, were purchased from a commercial meat supplier 3 d post slaughter (ps). The semimembranosus (SM), semitendinosus (ST), biceps femoris (BF) and rectus femoris (RF) muscles were dissected and closely trimmed of all connective tissue membranes and seam and intermuscular fat. The hams were treated as follows:

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

Ham 2 - Frozen. The dissected muscles were individually double-wrapped in freezer paper and held at -10°C for 8 d. On day 11 ps, 5-to-7 g of each muscle were analyzed for LDH activity.

Ham 2 - Frozen and Thawed. After obtaining frozen muscle samples from ham 2, the remaining muscles were wrapped singly in freezer paper and allowed to thaw for 24 hr at 4°C. Five-to-7 g samples of each muscle were analyzed for LDH activity.

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

Hams 4, 5, 6 - Heat-treated. Eleven-to-15 g samples were obtained from each of the four muscles of each fresh ham, placed in polycarbonate tubes and heated to specified endpoint temperatures in a constant temperature water bath. Samples from each muscle received nine heating time/temperature treatments:

| Temperature | Holding time |
|---|---|
| 65°C | 8, 23, 38 min |
| 69°C | 8, 23, 38 min |
| 71°C | 8, 23, 38 min |

The temperature was monitored using a 30 gauge copper-constantan insulated thermocouple wire positioned in the geometric center of each sample and attached to a Honeywell recorder. Core samples of 3-to-4 g, obtained from the geometric center of each sample, were retained for LDH activity analysis.

Hams 7, 8, 9 - Heat-treated and Cured. The SM and adductor (AD) muscles were designated as group A, while BF and ST were designated as group B, resulting in two test groups: A and B. Each muscle group was bisected allowing for one section to be tested uncured and the opposing section to be tested following cure application.

Using a needle and syringe, cured muscles were injected with the following brine formula:

0.1% sodium nitrite
1.25% sucrose
0.42% sodium erythorbate
0.5% sodium tripolyphosphate
15% sodium chloride

Following cure injection at 15% of the fresh weight, the muscles were held in a brine soak at 5°C for 65 hr. The brine soak consisted of the same mixture of ingredients as the injected brine, less the sodium

erythorbate. Muscle samples, weighing 11-to-15 g, were extracted using a 1.5 mm cork borer. Uncured samples were placed in polycarbonate tubes filled with double-distilled water and heated in a constant temperature water bath. The cured samples were placed in tubes containing the brine soak and heated to the same temperature endpoints.

Each muscle group was subjected to four heating time/temperature treatments:

| Temperature | Holding time |
|---|---|
| 65°C | 8, 23 min |
| 69°C | 8, 23 min |

The temperature of each sample was maintained at the desired endpoint for the appropriate holding time while being monitored with an Omega 871 digital thermometer and a 30 gauge chromel-alumel insulated thermocouple wire positioned in the geometric center of each muscle sample. Upon reaching the target temperature, heated samples were placed in Whirl Pak® bags and immediately submerged in ice water to halt the heating process. One-to-2.5 g samples were taken from the geometric center of each heated sample and prepared for LDH activity analysis.

Homogenate preparation. Following the prescribed treatment, 1-to-6 g samples of muscle were homogenized in a Virtis "45" homogenizer (speed 40, 30 sec) with a .01M potassium phosphate buffer. The homogenate was placed in a Beckman J2-21 centrifuge for 30 min at 13,823 x g. The fat was aspirated from the enzyme suspension and an aliquot of supernatant was diluted with .01M phosphate buffer to either a 320:1, 360:1 or 400:1 dilution. LDH activity of each sample was determined according to the analysis procedures of Vassault (1983), using a Beckman DU-7 spectrophotometer. Enzymatic determinations were performed five times for each treatment sample and were reported as the mean enzyme activity rate for that sample.

## RESULTS AND DISCUSSION

Muscles vary in their content and distribution of red and white fiber types as well as content of lactate dehydrogenase (Beecher, et al., 1965). White fibers contain primarily the M4 isozyme while the H4 isozyme functions predominantly in red fibers (Pesce et al., 1963; Pesce et al., 1966). The isozymes differ in effectiveness of their NADH utilization, therefore, when comparing LDH activities in different muscles, different activity rates result (figure 1). SM and RF muscles, from fresh porcine tissues and having predominantly red fibers, showed LDH activity rates of 742.3 and 753.4 umol/min x g, respectively. Lower activity rates of 652.8 and 460.7 umol/min x, respectively, were observed in ST and BF, muscles having primarily white fibers. However, no inferences can be drawn from comparisons of LDH activity between fiber types because ST and BF both have areas where either red or white fibers predominate and sampling was performed at random.

LDH activity in aged SM was 797.5 umol/min x g compared to 742.3 umol/min x g in the fresh sample (figure 1). ST muscle, however, exhibited slightly more LDH activity in the fresh sample compared to the aged, 652.8 and 548.8 umol/min x g, respectively. LDH activity in aged BF was 1,187 umol/min x g, a 61.2% increase in activity compared to 460.7 umol/min x g in the fresh sample. Aged RF exhibited 28.6% more LDH activity than did the fresh sample with rates of 1,055.4 and 753.4 umol/min x g, respectively. The reason for the notable increases in LDH activity of the BF and RF muscles is unknown, since each are representative of white and red muscles, respectively. However, sarcoplasmic protein hydrolysis during the aging period is postulated as the likely cause of the increased activity.

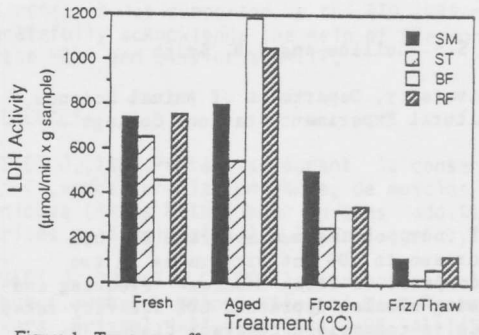


Figure 1. Treatment effects on lactate dehydrogenase activity in ham muscles. SM=Semimembranosus m., ST=Semitendinosus m., BF=Biceps femoris m., RF=Rectus femoris m.

In figure 1, the same pattern of LDH activity occurs in frozen tissue as that of fresh samples but at diminished activities for all four porcine muscles. LDH activity in fresh SM muscle dropped from 742.3 to 501.4 umol/min x g in frozen tissue, a decrease of 32.4%, while fresh ST muscle decreased 61.6% when frozen, from 652.8 to 250.9 umol/min x g. Likewise, BF LDH activity dropped 29.1% from 460.7 umol/min x g in fresh tissue to 326.6 umol/min x g in frozen tissue. This same trend was observed in RF with fresh and frozen tissue LDH activities of 753.4 and 350.0 umol/min x g, respectively, representing a 53.5% loss of activity. Freezing of muscle tissue results in a decrease in enzymatic activity which is likely due to ice crystal formation and subsequent rupture of cell membranes (Forrest et al., 1975). This allows the water soluble components, such as salts, amino acids, peptides and proteins to be lost from the tissue as drip.

Thawing frozen muscle tissue causes even more damage to the tissue than does freezing. During thawing, the meat temperature rises rapidly to the freezing point and remains at that point throughout the course of thawing. As a result, there is a greater opportunity for the formation of new, large ice crystals, increased microbial growth and for other chemical changes (Forrest et al., 1975). The freezing/thawing process results not only in increased damage to the muscle tissues and cell leakage, but also to increased protein denaturation and possibly loss of enzyme activity. LDH activity in all four muscles tested was consistently lower in the frozen/thawed samples when compared to the frozen muscles (figure 1). For example, LDH activities in frozen/thawed SM, ST, BF and RF samples were 119.1, 20.82, 64.76 and 124.4 umol/min x g, respectively. When compared to freezing alone, freezing/thawing resulted in a decline in LDH activity of 76.2, 91.7, 80.2 and 64.4% in SM, ST, BF and RF muscle, respectively.

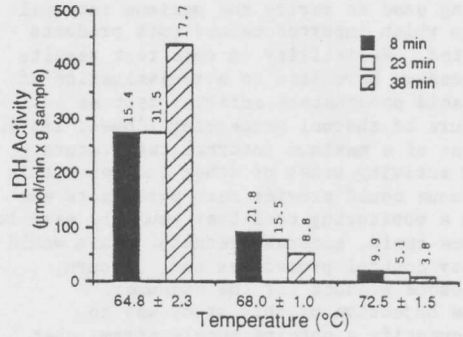


Figure 2. Composite lactate dehydrogenase activities measured in fresh semimembranosus, semitendinosus, biceps femoris and rectus femoris muscles heated to various endpoint temperatures and held for 8, 23 or 38 minutes.

Figure 2 illustrates an overall decrease in LDH activity in all four muscles of fresh hams when heated to specified temperatures between 62.0 and 74.0°C and held for 8, 23 or 38 min. As the muscles were exposed to increasing temperatures, enzymatic activity decreased. LDH activities at 65°C were 274.7, 275.3 and 438.8 umol/min x g for 8, 23 and 38 min, respectively. Holding the muscles at 65°C for 38 min increased the combined enzyme activities over that of 8 and 23 min, but these were still lower than most raw tissue activities. Temperature, therefore, 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 67°C resulted in LDH activities of 91.6, 78.4 and 54.0 umol/min x g for 8, 23 and 38 min or decreases of 66.6, 71.5 and 87.7%, respectively. At some point between 65°C and 68°C, the porcine muscle samples lost a significant amount of LDH activity regardless of the holding time. The loss of LDH activity between 68°C and 74°C was of much smaller magnitude comparatively, with minimal activities of 21.9, 18.1 and 9.5 umol/min x g for 8, 23 and 38 min, respectively.

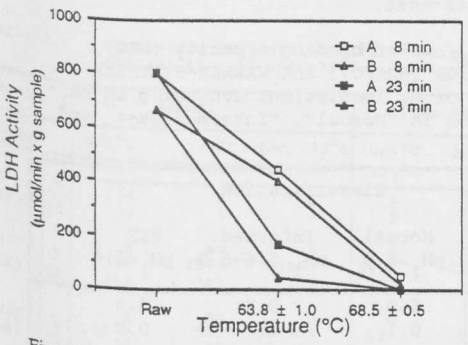


Figure 3. Lactate dehydrogenase activity levels measured in uncured ham muscles heated to various enpoint temperatures and held at those temperatures for 8 or 23 minutes. A=Semimembranosus m. and Adductor m., B=Biceps femoris m. and Semitendinosus m.

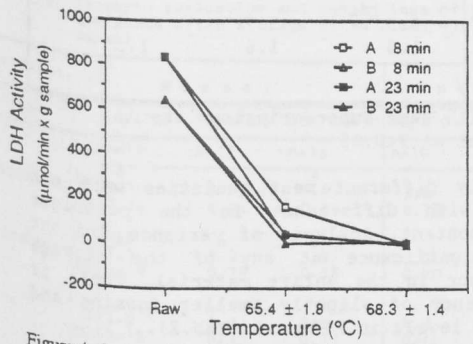


Figure 4. Lactate dehydrogenase activity measured in cured ham muscles heated to various endpoint temperatures and held at those temperatures for 8 or 23 minutes. A=Semimembranosus m. and Adductor m., B=Biceps femoris m. and Semitendinosus m.

In figures 3 and 4, LDH activity rates in the raw, uncured muscle halves of groups A (SM, AD) and B (BF, ST) were very similar to the rates in the opposing muscle halves that were unheated and cured. Initial LDH activities of raw muscle samples for group A and B were 800.2 and 669.4 umol/min x g, respectively, while cured samples values were 836.7 and 641.0 umol/min x g, respectively. Upon heating from 63 to 65°C for 8 min, however, LDH activity averaged 422.8 umol/min x g in uncured samples while in the cured muscle counterpart, the activity was only 107.3 umol/min x g. Increasing the temperature range from 68 to 69°C resulted in virtually no LDH activity in either the uncured or cured samples regardless of holding time. LDH activities of muscles A and B (figure 3) heated for 8 min at 63 to 65°C decreased by 44.1 and 40.5%, respectively, when compared to the raw tissue. However, when these tissues were heated for 23 min at 63 to 65°C, activities decreased by 79.0 and 93.7%, respectively.

Cured samples heated to 65.4 $\pm$ 1.8°C and held at those temperatures for 8 min showed an average decrease of 83.6% in activity when compared to the raw cured samples (figure 4). Likewise, a 97.4% rate decrease was observed in cured samples heated to 65.4 $\pm$ 1.8°C and held for 23 min compared to the raw cured samples. Regardless of the endpoint temperature achieved above 67°C or the time held at that temperature, the combined effects of curing and heating eliminated LDH activity prematurely. Since the LDH activity in ham muscles dropped dramatically when subjected to freezing, freezing/thawing and heating to temperatures below 68.9°C, this enzyme does not appear to be useful for monitoring the processing endpoint temperatures of cured, canned hams.

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