Antibody Development and Enzyme-Linked Immunosorbent Assay for the Protein Marker Lactate Dehydrogenase To Determine Safe Cooking End-Point Temperatures of Turkey Rolls

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An indirect competitive enzyme-linked immunosorbent assay (ELISA) was developed for determining the end-cooking temperature of turkey breast rolls. Electrophoresis of extracts from turkey rolls processed to internal temperatures between 68.3 and 72.1 °C revealed a single protein which disappeared from the extract at 70.9 °C. This protein was identified as lactate dehydrogenase (LDH) on the basis of molecular weight determination by sodium dodecyl sulfate electrophoresis, enzyme assay, and LDH-specific stain on native polyacrylamide gels. Polyclonal antisera were raised in rabbits against purified turkey muscle LDH and commercial chicken muscle LDH and yielded titers ranging from 3.6×10^5 to 1×10^6 after 10 weeks of immunization. Turkey muscle LDH antisera cross-reacted with chicken muscle LDH and vice versa but not with other species. The LDH content in precooked turkey breast roll extracts as determined by ELISA decreased as the end-cooking temperature increased, suggesting that the assay could serve as a simple and rapid method to determine adequacy of processing.

INTRODUCTION

Recent concern by meat processors, consumers, and the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) over the possibility of foodborne disease outbreaks from precooked meats contaminated by pathogens such as *Salmonella*, hemorrhagic *Escherichia coli*, *Campylobacter*, and *Staphylococcus* have prompted the need for rapid, accurate assays to verify that meat products receive sufficient heating to destroy these microorganisms.

The U.S. Department of Agriculture uses several assays to determine if precooked meat products have been cooked to the proper end-point temperatures as required in Title 9 of the Code of Federal Regulations (CFR). The Food Safety and Inspection Service describes procedures for a coagulation test (USDA, 1986a) for beef and pork products, a bovine catalase test (USDA, 1989), for roast beef and a residual acid phosphatase activity method (USDA, 1986b) for canned hams, picnics, and luncheon meats. Title 9 of the CFR requires precooked uncured poultry products to be cooked to an internal temperature of 71.1 °C; however, the USDA does not have a standard assay for verifying processing temperatures in poultry.

Several investigators have developed other enzyme assays to monitor end-point cooking temperatures based on the residual activity of catalase, peroxidase (Morozova and Soboleva, 1974), lactate dehydrogenase (LDH) (McCormick et. al., 1988; Stadler et al., 1991; Collins et al., 1991a,b), pyruvate kinase (Davis et al., 1988), glutamic-oxaloacetic transaminase, and glutamic–pyruvic transaminase (Townsend and Davis, 1990). These assays are very time-consuming or require a large number of reagents and/or sophisticated scientific equipment and thus have not been adopted for widespread use.

Several sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) techniques have also been evaluated for assessing the end-point cooking temperatures of meat products (Steele and Lambe, 1982; Lee et al., 1974; Alvarez, 1990). The loss of protein solubility has been used to measure the degree of heat denaturation of muscle proteins extracted from heated muscle with water or low ionic strength salt solutions (Lee et al., 1974; Davis et al., 1987; McCormick et al., 1987). These methods are accurate but time-consuming and not practical for routine use. A rapid qualitative assay for end-point cooking temperatures based on soluble protein composition is highly desirable.

Immunoassay are now being used to detect undeclared meat species, nonmeat proteins, microorganisms, mycotoxins, hormones, pesticides, and other contaminants in meat and meat products (Dincer et al., 1987; Sawaya et al., 1990; Fukal, 1991). Relatedly, enzyme-linked immunosorbent assays (ELISAs) have been developed to detect the presence of native and heat-denatured ovalbumin in food products (Breton et al., 1988, 1989). The goal of this project was to develop an ELISA to accurately determine if poultry breast rolls have been processed to the proper USDA end-point heating temperature. An indicator protein in turkey muscle extracts was identified and isolated. Polyclonal antibodies were raised in rabbits against this indicator protein, and an indirect competitive ELISA was devised to verify the end-cooking temperature of turkey rolls.

MATERIALS AND METHODS

Materials. Polyethylene sorbitan monolaurate (Tween 20), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide, and LDH from chicken muscle, chicken heart, bovine muscle, bovine heart, porcine muscle, porcine heart, and rabbit muscle were purchased from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin (BSA) (fraction V) was from Ameresco (Solon, OH). Goat anti-rabbit IgG conjugated to horseradish peroxidase was obtained from Cappel Laboratories (West Chester, PA). Complete and incomplete Freund's adjuvant were purchased from Difco Laboratories (Detroit, MI) and polystyrene microtiter ELISA plates (Immunolon-2 Removawells) from Dynatech Laboratories (Alexandria, VA). Rabbits (New Zealand white female) were obtained from the Bailey Rabbitry (Alto, MI). Commercial precooked turkey breast roasts and hams were purchased from a local retail store. All other chemicals were of reagent grade or better.

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Table I. Processing Schedule for Turkey Rolls

stage	time, min	internal temp, °C	dry bulb, °C	wet bulb, °C
1	60		60.0	60.0
2	120		65.6	65.6
3		66.7	71.1	71.1
4		70.0	73.9	73.9
5		72.2	75.6	75.6

Processing of Turkey Rolls. Turkey rolls were commercially prepared and formulated using 45.36 kg of turkey breast meat, 8.62 kg of water, 1.27 kg of modified starch, 0.68 kg of salt, 0.27 kg of sugar, and 0.23 kg of sodium tripolyphosphate. Product was stuffed in moisture-proof casings. Each roll weighed about 3.63 kg and measured 10.16 cm in diameter by 35.56 cm in length. Product was transported to Michigan State University and processed the next day.

Turkey rolls were smokehouse processed to target internal temperatures of 68.9, 70.0, and 71.1, and 72.2 °C using the smokehouse schedule in Table I. Internal product temperature was monitored by a thermocouple thermometer throughout the cooking and cooling cycle. Somkehouse runs were performed in triplicate. Turkey rolls were removed from the smokehouse approximately 1 °C before the desired internal temperature to allow for postprocessing temperature increases, cooled in an ice–water bath for 1.5 h, and then stored overnight at 4 °C before analysis.

Extraction of Protein from Turkey Rolls. A 3-cm slice of meat, immediately adjacent to a thermocouple location, was cut form the turkey roll. A 3 cm diameter core was cut from the center of the slice. Twenty-five grams of meat from this core sample was homogenized with 3 volumes (w/v) of cold phosphate buffer saline (PBS, 0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2) in a Waring blender for 90 s (three repetitions of 30 s on, 10 s off). The homogenate was centrifuged at 16000*g* for 20 min at 4 °C, the supernatant was filtered through Whatman No. 1 filter paper, and protein concentration was determined by the biuret method with BSA as the standard (Gornall et al., 1949). Percentage extractable protein was determined by dividing the protein content of the extract by the protein content of the turkey rolls and multiplying by 100.

Electrophoresis. To determine protein composition of the meat extracts, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (Laemmli, 1970) or native PAGE (Davis, 1964) was performed using either a Mini-Protean II electrophoresis unit (Bio-Rad Laboratories, Richmond, CA) or a Hoeffer vertical electrophoresis unit (Model SE 600) and a Bio-Rad power supply (Model 1000/500). For SDS–PAGE, stacking and separating gels of 4 and 12% acrylamide, respectively, were used. Molecular weights were determined on SDS–PAGE gels by comparing relative mobilities of protein bands to those of molecular weight standards (SDS-6H and SDS-7, Sigma) (Weber and Osborn, 1969). Protein bands were stained with Coomassie Brilliant Blue R 250 or silver stain (Merril, 1990).

The native PAGE separating gel (pH 8.8) was made up of 4.5% acrylamide and covered with a thin layer (5 mm) of 3% acrylamide gel (pH 8.8). Native PAGE gels were stained for the presence of LDH using an LDH-specific staining solution based on the methods of Fine and Costello (1963) and Fritz et al. (1970). One hundred milliliters of the stain contained 0.1 M Tris, pH 8.5, 0.02 M lithium lactate, 35.3 mg of NAD^+, 17.7 of mg of nitro blue tetrazolium chloride, and 0.7 mg of phenazine methosulfate. The gel was stained at 25 °C for 1 h in the dark, and the reaction was stopped by soaking the gels in 7.0% v/v acetic acid.

Determination of LDH Activity. Activity was determined using an LDH diagnostic kit (DG 1340-K, Sigma) at 25 °C. One unit of LDH activity was expressed as 1 μmol of NADH oxidized per minute.

Purification of LDH from Turkey Breast. Turkey LDH was purified on the basis of the recommendations of Scopes (1970). Turkey breast was ground through the 4-mm plate of a KitchenAid grinder (Hobart Corp., Troy, OH) and then homogenized with 2 volumes (w/v) of cold distilled water in a Waring blender for 30 s. The homogenate was stirred for 30 min at 4 °C and filtered through gauze. The filtrate was adjusted to pH 5.0 with 1 M acetic acid and centrifuged at 16000*g* for 20 min at 4 °C. The supernatant was heated to 65 °C in a water bath and then cooled immediately in an ice–water bath. The heated solution was centrifuged at 16000*g* for 45 min at 4 °C. The supernatant was brought to 0.80 saturation with solid ammonium sulfate and then centrifuged at 16000*g* for 20 min at 4 °C. The precipitate was collected and dissolved in 0.15 M acetate buffer (pH 5.0). This solution was heated to 63 °C in a water bath, cooled in an ice–water bath, and then centrifuged at 16000*g* for 20 min. The precipitate was discarded, and the ammonium sulfate saturation of the supernatant was raised to 0.45 with saturated ammonium sulfate solution and left at 4 °C overnight. The solution was centrifuged as described above and the precipitate discarded. The ammonium sulfate saturation was raised to 0.60 and held at 4 °C for crystallization of LDH. This turbid solution was centrifuged at 16000*g* for 20 min. The precipitate was dissolved in distilled water and dialyzed against distilled water. The dialysate was cooled to about 0 °C, cold acetone (−10 °C) was added to 40% acetone concentration (v/v), and the mixture was left to stand at −7 °C for 30 min. This solution was centrifuged at 4000*g* for 15 min at −7 °C. The precipitate was dissolved in PBS and dialyzed against PBS.

Production of Polyclonal Antibodies. Rabbits (three per group) were injected subcutaneously with 500 μg of purified turkey muscle LDH or commercial chicken muscle LDH mixed with saline–Freund's complete adjuvant (1:1) in a volume of 1.0 mL. Five weeks later these were boosted by subcutaneous injection with 500 μg of enzyme in saline–Freund's incomplete adjuvant (1:1) in a volume of 1.0 mL. At week 8, rabbits were boosted intramuscularly with 200 μg of enzyme in 1:1 saline–Freund's incomplete adjuvant. Rabbits were bled via marginal veins at intervals and sacrificed 10 weeks after initial immunization. Antibodies were purified by ammonium sulfate (33% saturation) (Hebert et al., 1973).

Indirect ELISA. For antisera titration, microtiter wells were coated overnight (4 °C) with 100 μL of LDH (5 μg/mL) in 0.1 M carbonate buffer (pH 9.6). Plates were washed four times with PBS containing 0.05% (v/v) Tween 20 (PBS–Tween). Wells were incubated with 300 μL of 1% BSA (w/v) in PBS (BSA–PBS) at 37 °C for 30 min to minimize nonspecific binding. After washing, 50 μL of serially diluted serum was added to each well and incubated for 1 h at 37 °C. Unbound antibody was removed by washing four times, and 100 μL of goat antirabbit IgG peroxidase conjugate (1:500 in 1% BSA–PBS) was added to each well. Plates were incubated for 30 min at 37 °C and washed eight times, and bound peroxidase was determined with ABTS substrate as described by Pestka et al. (1982). Absorbance was read at 405 nm using a Minireader II (Dynatech), and the titer of each serum was arbitrarily designated as the maximum dilution that yielded at least twice the absorbance of the same dilution of nonimmune control serum.

A competitive ELISA was used to test the ability of LDH antibodies for detection of LDH in processed turkey and chicken meat products. The competitive assay was essentially identical with titer determination, except that, after BSA blocking and washing, 50 μL of standard LDH or meat extract was added to each well followed by 50 μL of the appropriate dilution of LDH antisera. Plates coated with turkey or chicken LDH were stored at 4 °C for at least 8 weeks without loss of activity and were used to determine sera titer and detect and quantify the amount of LDH in turkey or chicken products. Sera of all six rabbits were used throughout this study with a dilution ranging from 1:50 000 to 1:150 000.

Western Blot Analysis. Purified turkey LDH and turkey roll extract proteins were transferred electrophoretically (1 h at 100 V) from the SDS–PAGE gel to nitrocellulose membrane (0.45 μm, Schleicher & Schuell, Keene, NH) in a Mini Trans-Blot unit (Bio-Rad) using 25 mM Tris, 192 mM glycine, and 20% (v/v) methanol buffer (pH 8.3). After transferring, the membrane was washed with PBS–Tween, blocked with 10 mL of 3% BSA–PBS for 30 min at room temperature, and rinsed with PBS–Tween and 10 mL of the appropriately diluted antibody in 3% BSA–PBS incubated with the membrane at room temperature for 30 min. Unbound antibody was removed by washing with PBS–Tween, and 10 mL of goat anti-rabbit IgG peroxidase conjugate (1:2000 in 3% BSA–PBS) was added to the membrane and

Table II. Effect of End-Point Cooking Temperature on Lactate Dehydrogenase Activity and Extractable Protein Content of Turkey Roll

internal temp, °C	extractable protein,[a] %	LDH activity,[a] units/g of sample
unheated	25.3 ± 0.2[b]	734.68 ± 44.04[b]
68.3 ± 0.3	3.8 ± 0.08[c]	22.04 ± 12.16[c]
69.7 ± 0.3	3.6 ± 0.03[cd]	2.28 ± 0.35[d]
70.9 ± 0.3	3.4 ± 0.02[d]	1.35 ± 0.09[d]
72.1 ± 0.3	3.5 ± 0.04[d]	1.18 ± 0.06[d]

[a] Expressed as mean ± standard deviation of three replicate values. Means in the same column followed by the same letter are not different ($P > 0.05$).

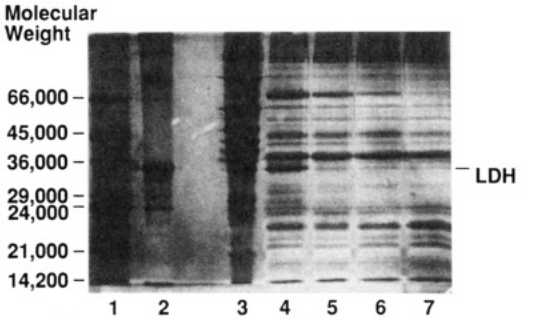

Figure 1. Representative sodium dodecyl sulfate–polyacrylamide gel electrophoretogram of muscle extracts from turkey rolls heated to different end-point temperatures. Proteins were visualized with silver stain. (Lane 1) Molecular weight marker; (lane 2) chicken muscle LDH (from Sigma); (lane 3) unheated turkey roll; (lane 4) 68.3 °C; (lane 5) 69.7 °C; (lane 6) 70.9 °C; (lane 7) 72.1 °C. Twenty micrograms of protein was loaded on each gel lane.

incubated at room temperature for 10 min. The membrane was washed with PBS–Tween, and bound peroxidase was determined with 15 mL of substrate solution (24 mg of 3,3′,5,5′-tetramethylbenzidine and 80 mg of dioctyl sulfosuccinate dissolved in 10 mL of ethanol, 30 mL of 0.1 M citrate–phosphate buffer, pH 5.0, and 20 μL of 30% H_2O_2) at room temperature. Staining was stopped by washing with water.

Statistics. Experiments with turkey rolls and commercial turkey products were performed in triplicate. Basic statistics and two-way analysis of variance (treatment × replication) were performed using MSTAT software (version C, 1989, Michigan State University, East Lansing, MI). Mean separations were performed using Tukey's test with the mean square error term at the 5% level of probability.

RESULTS AND DISCUSSION

Average maximum internal temperatures of the turkey rolls were 68.3, 69.7, 70.9, and 72.1 °C, which corresponded to target temperatures of 68.9, 70.0, 71.1, and 72.2 °C, respectively. Extractable protein decreased from 25.3% in the unheated rolls to an average of 3.6% at internal temperatures between 68.3 and 72.1 °C (Table II). Extractable protein content did not differ ($P > 0.05$) in turkey breast rolls processed between 68.3 and 72.1 °C.

A representative SDS–PAGE electrophoretogram of meat extracts from turkey rolls processed to different end-point temperatures is shown in Figure 1. Extracts from unheated turkey rolls had over 17 bands. Fewer bands were observed in the heated samples. Band patterns of extracts from meat processed to 68.3 °C were not different from those of meat processed at 69.7 °C except for a decrease in the intensity of a 35 000-Da band. The intensity of this band decreased further as processing temperature was increased to 70.9 °C. A protein band with molecular weight of 66 000 was observed in all extracts but decreased markedly in extracts from turkey rolls processed to 72.1 °C. The 35 000- and 66 000-Da bands

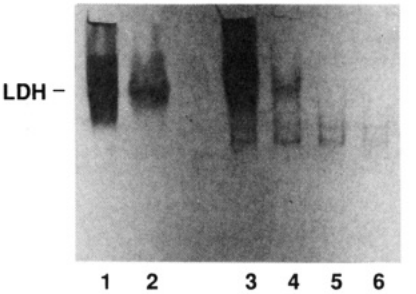

Figure 2. Representative native polyacrylamide gel electrophoretogram with LDH-specific stain of muscle extracts from turkey rolls heated to different end-point temperatures. (Lane 1) Unheated turkey roll; (lane 2) isolated turkey muscle LDH; (lane 3) 68.3 °C; (lane 4) 69.7 °C; (lane 5) 70.9 °C; (lane 6) 72.1 °C. Twenty micrograms of protein was loaded on each gel lane.

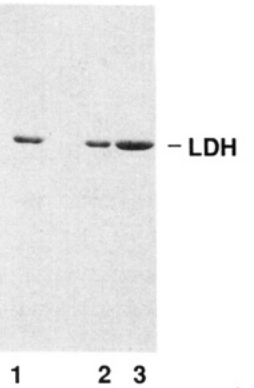

Figure 3. Representative sodium dodecyl sulfate–polyacrylamide gel electrophoretogram of chicken and turkey breast muscle lactate dehydrogenase (LDH). (Lane 1) Chicken muscle LDH (from Sigma); (lanes 2 and 3) isolated turkey muscle LDH. Twenty micrograms of protein was loaded on each gel lane. Gels were stained with Coomassie Blue.

were presumptively identified on the basis of molecular weight as a subunit of LDH and monomeric phosphoglucomutase, respectively (Scopes, 1970).

On native PAGE, bands with the same mobility as isolated turkey muscle LDH (lane 2) were observed in unheated turkey rolls (lane 1) and in turkey rolls processed to 68.3 (lane 3) and 69.7 °C (lane 4) but not in rolls processed to 70.9 (lane 5) or 72.1 °C (lane 6) (Figure 2). LDH activity in the extracted fraction decreased from 22.0 units/g at 68.3 °C to 2.3 units/g at 69.7 °C. LDH activity at 70.9 and 72.1 °C was not different ($P > 0.05$) and averaged 1.3 units/g of meat. Using reversed-phase high-performance liquid chromatography, McCormick et al. (1987) also showed decreased LDH content in porcine muscle extracts between 65 and 70 °C.

Since LDH was not detected on native gels using a specific enzyme stain and activity was negligible in extracts of turkey rolls processed to internal temperatures of 70.9 °C, this protein was selected as the indicator protein for immunochemical verification of safe end-point cooking temperatures of turkey breast products. The isolated turkey breast muscle LDH migrated the same distance as a commercial chicken muscle LDH on SDS–PAGE (Figure 3) and had an enzyme activity of 300 units/mg of protein.

For generation of specific antibodies for an LDH assay, rabbits were immunized with commercial chicken LDH or with purified turkey LDH. Antibody titers were detected in the sera of the immunized rabbits as early as 4 weeks after the initial immunization (Table III). The antibody titer rose sharply at week 7 following one booster injection and reached a maximum at week 10 following a second booster injection. Figures 4 and 5 contain titra-

Table III. Production of Polyclonal Antibodies against Lactate Dehydrogenase (LDH) of Chicken or Turkey Muscles in Rabbits[a]

weeks after immunization[b]	antibody titer					
	chicken LDH			turkey LDH		
	A	B	C	D	E	F
4	6×10^3	1.2×10^4	2.5×10^4	1.0×10^5	2.0×10^5	2.5×10^4
7	2.1×10^5	3.5×10^5	1.8×10^5	3.6×10^5	6.8×10^5	2.1×10^5
10	5.1×10^5	8.1×10^5	3.5×10^5	7.1×10^5	1.0×10^6	5.4×10^5

[a] A, B, and C refer to rabbit antisera immunized with chicken LDH. D, E, and F refer to rabbit antisera immunized with turkey LDH. [b] Booster injections were performed at weeks 5 and 8.

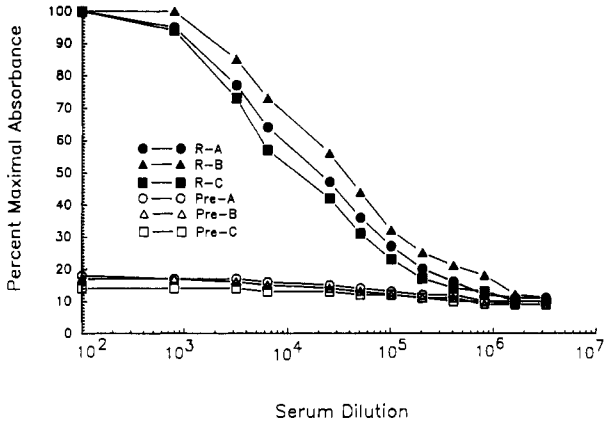

Figure 4. ELISA titration of rabbit anti-chicken lactate dehydrogenase antibodies. Rabbit antisera R-A, R-B, and R-C were obtained 10 weeks after initial immunization. PRE indicates preimmune serum.

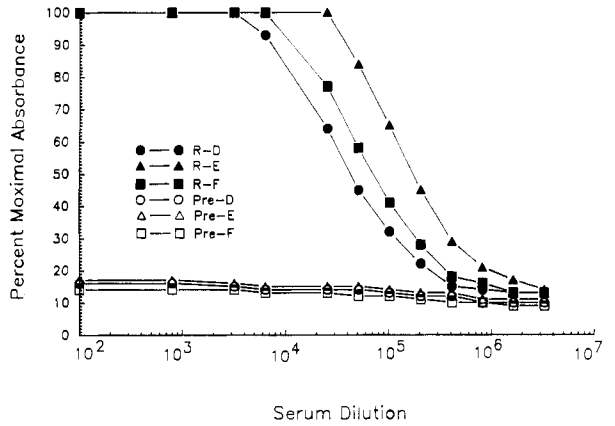

Figure 5. ELISA titration of rabbit anti-turkey lactate dehydrogenase antibodies. Rabbit antisera R-D, R-E, and R-F were obtained 10 weeks after initial immunization. PRE indicates preimmune serum.

tion curves for rabbits immunized with chicken LDH and turkey LDH, respectively. Generally, higher titers were observed from rabbits injected with turkey LDH compared to those injected with chicken LDH.

A competitive indirect ELISA (CI-ELISA) was devised using turkey or chicken LDH as solid phase/inhibitor pairs (Figure 6). It was determined that antibodies prepared against turkey LDH were more effective than antibodies prepared against chicken LDH regardless of the solid phase/inhibitor pair. The specificity of the polyclonal antibodies in CI-ELISA was further examined by evaluating reactivity with LDH from rabbit, bovine, and porcine skeletal muscle and bovine, porcine, and chicken heart (Figures 7 and 8). None of these enzymes inhibited binding of either turkey or chicken LDH antibodies up to concentrations of 25 000 ng/mL, thus verifying assay specificity for turkey and chicken muscle LDH.

Western blot analysis revealed that polyclonal antibodies

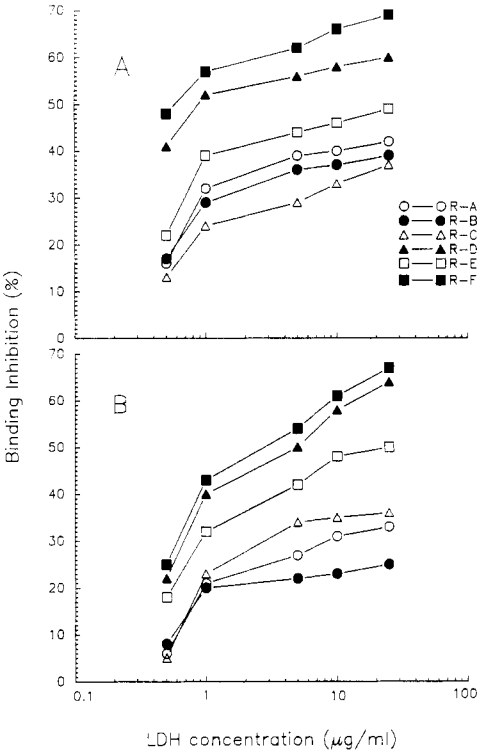

Figure 6. ELISA standard curves of rabbit polyclonal antibodies prepared against chicken lactate dehydrogenase (LDH) (R-A, R-B, and R-C) or turkey LDH (R-D, R-E, and R-F). (A) Turkey LDH was used as solid phase and as inhibitor in the competitive ELISA; (B) chicken LDH was used as solid phase and inhibitor.

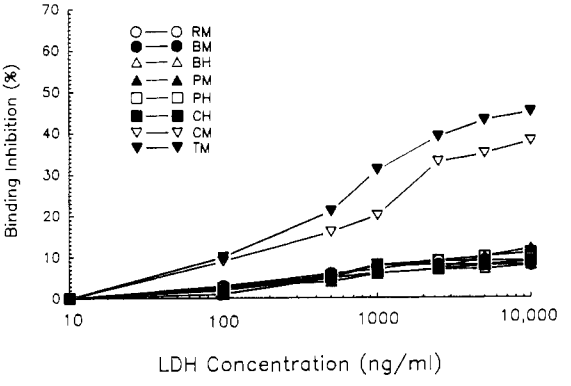

Figure 7. Representative graph of cross-reactivity of chicken lactate dehydrogenase (LDH) polyclonal antibodies with LDH from different animal sources: rabbit muscle (RM), bovine muscle (BM), bovine heart (BH), porcine muscle (PM), porcine heart (PH), chicken heart (CH), chicken muscle (CM), and turkey muscle (TM). Serum of rabbit F was used.

to turkey LDH reacted with purified turkey muscle LDH and with LDH processed turkey roll extracts (Figure 9). The intensity of the LDH band identified by Western blot decreased as end-point heating temperature of the turkey rolls increased. Some binding was observed to

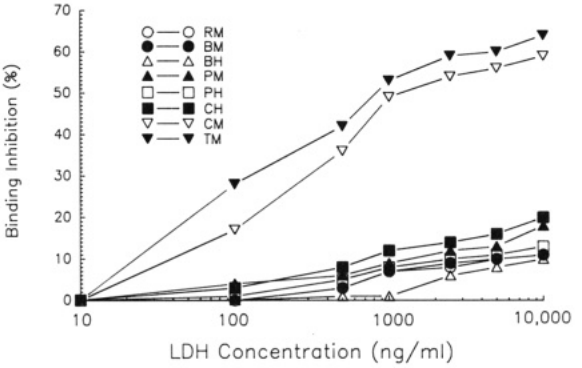

Figure 8. Representative graph of cross-reactivity of turkey lactate dehydrogenase (LDH) polyclonal antibodies with LDH from different animal sources. Abbreviations of LDH source are the same as in Figure 7. Serum of rabbit F was used.

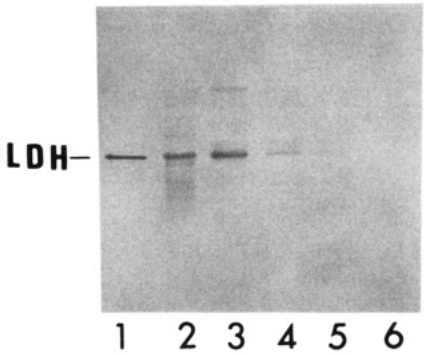

Figure 9. Western blot of isolated turkey muscle LDH and muscle extracts from turkey rolls heated to different end-point temperatures. (Lane 1) Isolated turkey muscle LDH; (lane 2) unheated turkey roll; (lane 3) 68.3 °C; (lane 4) 69.7 °C; (lane 5) 70.9 °C; (lane 6) 72.1 °C. The amounts of protein loaded on the gel lane were 0.07, 1.2, and 20 μg for isolated turkey muscle LDH, unheated turkey roll, and heated turkey rolls, respectively.

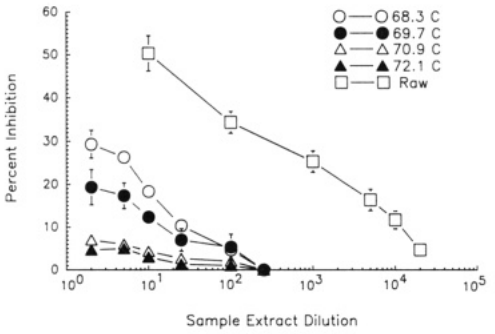

Figure 10. Indirect competitive ELISA to determine the cooking end-point by measuring LDH in turkey rolls cooked to different processing temperatures. Data are the average of triplicate samples. Bars indicate standard error of the mean. Data points without bars indicate error bars fit within points.

protein bands other than turkey muscle LDH, which may be attributed to nonspecific protein binding, the presence of other LDH isozymes, or very slight cross-reactivity of the polyclonal antibodies with proteins other than LDH.

The LDH in turkey extracts decreased as the end-cooking temperature increased as determined by CI-ELISA (Figure 10). Percent inhibition for extracts diluted 2–5-fold exceeded 10% for raw turkey and samples heated to 68.6 and 69.7 °C but dropped below 10% for samples heated to 70.9 and 72.1 °C. The results indicated that LDH was virtually absent at these latter two temperatures and suggested that a simple ELISA could be used to assess whether the USDA-required safe cooking end-point temperature of 71.1 °C was reached.

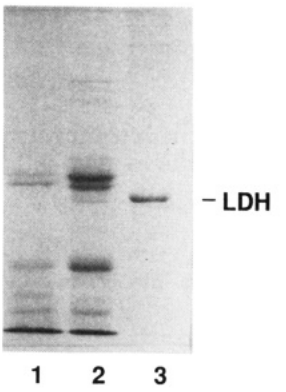

Figure 11. Representative sodium dodecyl sulfate–polyacrylamide gel electrophoretogram of muscle extracts of commercial turkey breast roast and turkey ham. (Lane 1) Turkey breast roast; (lane 2) turkey ham; (lane 3) chicken LDH (from Sigma). Twenty micrograms of protein was loaded on each gel lane.

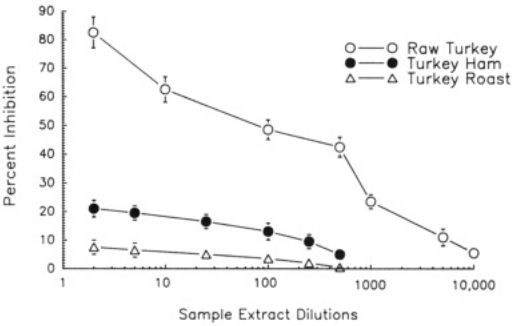

Figure 12. Indirect competitive ELISA of commercial turkey breast roast and turkey ham extracts. Extracts were diluted in 1% bovine serum albumin in phosphate buffer saline (PBS–BSA). Turkey was extracted 1:3 (w/v) and then diluted further in 1% PBS–BSA. Bars indicate standard error of the mean. Data points without bars indicate error bars fit within points.

Commercially processed turkey breast roast and turkey ham were tested for the presence of LDH by SDS–PAGE, enzyme assay, and CI-ELISA. As shown on electrophoretograms (Figure 11), LDH was not observed in turkey breast roast (lane 1) but was observed in turkey ham (lane 2). LDH activities for turkey breast roast and turkey ham were 1.76 and 33.44 units/g of sample, respectively. These results agreed with the indirect competitive ELISA (Figure 12). Percent inhibition for turkey ham was greater than 10 within the dilution range 2–10-fold, indicating the internal temperature did not reach 71.1 °C. In contrast, inhibition for turkey breast roast was below 10%, indicating the adequacy of heat processing. Since turkey ham is processed to a lower temperature (68.3 °C) than turkey breast products, more LDH was detected by CI-ELISA.

In summary, we have identified LDH as an indicator protein and demonstrated the feasibility of using ELISA to determine the end-cooking temperature of turkey rolls. ELISA accurately differentiated the end-cooking temperature within ±1.1–1.2 °C in the temperature range 68.3–72.1 °C. Although LDH activity assay was consistent with ELISA, it was very difficult to set a critical value of LDH activity for the adequacy of heat treatment. LDH-specific stain and native PAGE were very sensitive for determining the end-cooking temperature but were more complicated than ELISA and the LDH activity assay. ELISA can screen a large number of samples at the same time by using 96-well microtiter plates, and the resulting color in ELISA can be measured spectrophotometrically or by visual comparison with standards. Thus, ELISA could serve as a simple and rapid method to determine adequacy of processing of turkey rolls. Experiments are in progress

to validate the assay under a range of ingredient formulations and processing conditions.

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