Lactate Dehydrogenase, Serum Protein, and Immunoglobulin G Content of Uncured Turkey Thigh Rolls As Influenced by Endpoint Cooking Temperature

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The efficacy of using a lactate dehydrogenase (LDH) sandwich enzyme-linked immunosorbent assay (ELISA), a turkey serum protein sandwich ELISA, and an immunoglobulin G (IgG) competitive indirect ELISA for determining the endpoint cooking temperature of uncured turkey thigh rolls was studied. Serum protein content in extracts was assessed by ELISA and was found to decrease as the cooking temperature increased. Serum protein ELISA differentiated between products cooked to 68.9 and 71.1 °C. Five LDH isozymes were found in raw turkey thigh rolls. In contrast to turkey breast rolls, the LDH ELISA could not differentiate the endpoint temperatures of thigh rolls processed between 68.9 and 71.1 °C due to the presence of three heat-stable isozymes. The IgG content was low and did not differ in turkey thigh rolls processed between 68.9 and 72.2 °C.

Keywords: *ELISA; endpoint temperature; turkey thigh*

INTRODUCTION

Title 9 of the Code of Federal Regulations requires thermal treatment of meat products to ensure the destruction of harmful microorganisms and viruses. The possibility of food-borne disease outbreaks from precooked meats contaminated by pathogens such as *Salmonella*, *Escherichia coli* 0157:H7, and *Listeria monocytogenes* is of concern to meat processors, consumers, and the U.S. Department of Agriculture Food Safety and Inspection Service (USDA–FSIS). Rapid and accurate methods are needed to verify the adequacy of thermal treatment of meat products.

Presently, the USDA–FSIS uses a residual phosphatase assay (USDA–FSIS, 1986a), a catalase test (USDA–FSIS, 1989), or a coagulation test (USDA–FSIS, 1986b) to verify endpoint cooking temperatures of beef and pork. These assays are time-consuming, empirical, and subjective (Townsend and Blankenship, 1989). When cooked under USDA–FSIS inspection, uncured poultry products are required to be cooked to an internal temperature of 71.1 °C (USDA–FSIS, 1992); however, the USDA does not have a standard assay for verifying processing temperatures in poultry.

The loss of solubility of water-soluble or sarcoplasmic proteins (primarily glycolytic enzymes) is related to heating time, endpoint temperature, and initial concentration of soluble proteins in muscle (Davis and Anderson, 1984). Residual enzyme activity has been correlated to internal processing temperature of meat products in several studies (Davis et al., 1988, 1991; Collins et al., 1991a,b; Bogin et al., 1992; Townsend and Davis, 1992; Kormendy et al., 1992; Townsend et al., 1993; Ang et al., 1993; Hsu et al., 1993). It has been reported that lactate dehydrogenase (LDH) activity could be used as an endpoint processing indicator in beef, pork, and turkey muscle (Collins et al., 1991a,b; Stalder et al., 1991; Bogin et al., 1992; Townsend and Davis, 1991; Hsu et al., 1993). McCormick et al. (1987) also showed that LDH content in porcine muscle extracts decreased between 65 and 70 °C.

Our laboratory has previously shown that LDH concentration can be used as an endpoint cooking indicator in commercially prepared turkey breast rolls (Wang et al., 1992). A competitive indirect enzyme-linked immunosorbent assay (CI-ELISA) (Wang et al., 1992) and sandwich ELISA (Abouzied et al., 1993) were developed to detect LDH in turkey breast rolls. Both ELISAs accurately differentiated the endpoint cooking temperature within ±1.1–1.2 °C between 68.3 and 72.1 °C. Salt concentration, cooking schedule, and product casing diameter of turkey breast rolls did not have a marked influence on sandwich LDH ELISA in determining endpoint cooking temperature; a maximum concentration of 0.31 μg of LDH/g meat was found to indicate proper processing of turkey breast rolls (Wang et al., 1993).

Townsend and Davis (1991) and Townsend et al. (1993) reported that LDH from dark meat was more heat stable than that from light meat. Thus, we questioned whether the above-described ELISA was applicable to dark meat. The purpose of this study was to investigate the feasibility of using ELISAs for LDH and various serum proteins to monitor the endpoint cooking temperature of uncured turkey thigh rolls. As turkey thigh muscle contains different LDH isozymes from those of breast muscle, the ability of LDH antibodies to detect different LDH isozymes was also studied.

MATERIALS AND METHODS

Materials. Polyoxyethylenesorbitan monolaurate (Tween 20), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), gelatin (type A, from porcine skin), turkey serum proteins, turkey serum albumin (TSA), chicken immunoglobulin G (IgG), polyclonal antibodies to turkey serum proteins (PAb-TS), polyclonal antibodies to turkey IgG whole molecule (PAb-IgG), biotin–amidocaproate *N*-hydroxysuccinimide ester, dimethyl sulfoxide, and avidin peroxidase conjugate were purchased from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin (BSA) (fraction V) was from Ameresco (Solon, OH). Goat anti-rabbit and anti-mouse IgGs conjugated to horseradish peroxidase were obtained from Cappel Laboratories (West

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Table 1. Processing Schedule for Turkey Thigh Rolls[a,b]

stage	cycle time (min)	internal temp (°C)	dry bulb temp (°C)	wet bulb temp (°C)
1	60	(30.8 ± 2.0)	60.0	60.0
2	120	(56.3 ± 0.3)	65.6	65.6
3	60	(61.6 ± 0.6)	71.1	71.1
4	60	(67.0 ± 0.5)	75.6	75.6
5	(73 ± 7)	71.7	79.4	79.4

[a] Total cooking time for turkey rolls processed to an internal temperature of 72.2 °C was about 6.5 h. [b] Values in parentheses indicate mean ± standard deviation of internal product temperature at the end of a cycle or cycle time to reach internal product temperature of 71.1 °C.

Chester, PA). Microtiter plates (Immunolon 2 Removawells) were from Dynatech Laboratories (Alexandria, VA). Turkey breast muscle LDH was purified using ammonium sulfate and acetone precipitation (Wang et al., 1992). All other chemicals were of reagent grade or better.

Conjugation of Biotin to PAb-TS. PAb-TS was purified using a protein G column according to the manufacturer's instructions (Pierce, Rockford, IL) and conjugated to biotin (Harlow and Lane, 1988). Two milliliters of antibody solution (3 mg/mL) was prepared in 0.1 M sodium borate buffer (pH 8.8). Biotin–amidocaproate *N*-hydroxysuccinimide ester was dissolved in dimethyl sulfoxide to 5 mg/mL, and then 0.12 mL of biotin ester solution was added dropwise to the antibody solution (0.1 mg of biotin ester/mg of antibody) with stirring. After incubation at 25 °C for 4 h, 48 μL of 1 M NH_4Cl (0.08 μL of 1 M NH_4Cl/μg of biotin ester) was added and incubated at 25 °C for 10 min. The final antibody solution was dialyzed against PBS.

Processing of Turkey Thigh Rolls. Turkey rolls were formulated using 45.4 kg of fresh skinless turkey thigh meat, 7.4 kg of water, 1.23 kg of NaCl, 0.73 kg of sugar, and 0.23 kg of sodium tripolyphosphate (Rhone-Poulenc, Washington, PA). Turkey thigh rolls were processed according to the method of Wang et al. (1993) except that ground meat was mixed with all of the ingredients for 25 min after turkey thigh meat was ground though a 24 × 28 mm kidney plate (Model 4146, Hobart Manufacturing Co., Troy, OH). Turkey thigh rolls were smokehouse processed using the schedule in Table 1. Each roll weighed about 3.63 kg and measured 10.16 cm in diameter by 35.56 cm in length. Calibration of recorders and probes was described previously (Wang et al., 1993). Smokehouse runs were performed in triplicate. The moisture, protein, and fat contents of turkey thigh rolls were 73.5 ± 0.31, 16.2 ± 0.11, and 3.9 ± 0.12%, respectively (AOAC, 1990); the pH of turkey rolls was 6.3 ± 0.02.

Extraction of Protein and LDH Activity. Meat (25 g) was excised from the geometric center of each turkey roll, adjacent to a thermocouple location, and homogenized and extracted in 0.15 M NaCl and 0.01 M sodium phosphate buffer, pH 7.2 (PBS) (Wang et al., 1992). Protein concentration of extracts was determined according to 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. The LDH activity in each extract 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/min.

Electrophoresis and Western Blot Analysis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and native PAGE were performed as described by Wang et al. (1992). SDS–PAGE gels were stained with Coomassie Brilliant Blue R 250. Native PAGE gels were stained for the presence of LDH using an LDH-specific staining solution (Wang et al., 1992).

To determine the binding ability of antibodies to LDH isozymes, turkey roll extract proteins were transferred electrophoretically (1 h at 100 V) from the native gel to a nitrocellulose membrane (0.45 μm, Schleicher and Schuell, Keene, NH) using 25 mM Tris and 192 mM glycine buffer, pH 8.3, in a Mini Trans-Blot unit (Bio-Rad Laboratories, Richmond, CA). After transferring, the membrane was washed with PBS containing 0.05% (v/v) Tween 20 (PBS–Tween), blocked with 10 mL of 3% (w/v) BSA in PBS (BSA–PBS) for 30 min at 25 °C, and rinsed with PBS–Tween. Ten milliliters of polyclonal LDH antibody F (1/2000) (Wang et al., 1992) or monoclonal LDH antibody D5E (1/400) (Abouzied et al., 1993) diluted in 3% BSA–PBS was added to the membrane and incubated at 25 °C for 30 min. Unbound antibody was removed by washing with PBS–Tween, and 10 mL of goat anti-rabbit IgG peroxidase conjugate (for polyclonal antibody) or goat anti-mouse IgG peroxidase conjugate (for monoclonal antibody) diluted (1/2000) in 3% BSA–PBS was added to the membrane and incubated at 25 °C for 10 min. The membrane was washed with PBS–Tween, and bound peroxidase was determined (Wang et al., 1992).

To detect turkey serum proteins or IgG in meat extracts, turkey roll extract proteins were transferred electrophoretically (1 h at 100 V) from the SDS–PAGE gel to a nitrocellulose membrane 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 1% gelatin (w/v) in PBS (gelatin–PBS) for 30 min, and rinsed with PBS–Tween. Ten milliliters of PAb-TS or PAb-IgG diluted (1/1000) in gelatin–PBS was added to the membrane and incubated for 30 min. Unbound antibody was removed by washing with PBS–Tween, and 10 mL of goat anti-rabbit IgG peroxidase conjugate diluted (1/2000) in gelatin–PBS was added to the membrane and incubated for 10 min. The membrane was washed with PBS–Tween, and bound peroxidase was determined (Wang et al., 1992).

Sandwich ELISA for LDH and Serum Protein. LDH sandwich ELISA was performed according to the method of Wang et al. (1993) using antibodies against turkey and chicken muscle LDH (Wang et al., 1992; Abouzied et al., 1993). Purified turkey LDH was used to prepare a standard curve in each plate. Results were expressed as nanograms of LDH per gram of turkey roll.

For serum protein ELISA, microtiter wells were coated with 100 μL of PAb-TS diluted (1/3000) in 0.1 M carbonate buffer, pH 9.6, and dried overnight at 40 °C in a forced air oven. Wells were washed four times with PBS–Tween, and 300 μL of 1% gelatin (w/v) in PBS–Tween (gelatin–PBS–Tween) was added to each well and incubated for 30 min at 37 °C to minimize nonspecific binding. After three washings with PBS–Tween, muscle extracts or standard TSA diluted in gelatin–PBS–Tween (100 μL) was added and incubated at 37 °C for 1 h. Plates were washed four more times with PBS–Tween, and 100 μL of biotin-labeled PAb-TS diluted (1/500) in gelatin–PBS–Tween was added. After incubation for 1 h at 37 °C and four washings with PBS–Tween, 100 μL of avidin peroxidase conjugate diluted (1/1500) in gelatin–PBS–Tween was added to each well. Plates were incubated for 30 min at 37 °C and washed eight times with PBS–Tween. Bound peroxidase activity was determined with ABTS substrate (Pestka et al., 1982) at 405 nm using a Minireader II (Dynatech, Alexandria, VA). Standard turkey serum proteins were used to prepare a standard curve in each plate, and results were expressed as milligrams of turkey serum proteins per gram of turkey roll.

Competitive Indirect ELISA for IgG. Microtiter wells were coated overnight (4 °C) with 100 μL of chicken IgG (5 μg/mL) in 0.1 M sodium borate 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 gelatin–PBS at 37 °C for 30 min. After three washings with PBS–Tween, 50 μL of meat extract diluted in PBS–gelatin was added to each well followed by 50 μL of PAb–IgG (1/5000) diluted in gelatin–PBS. After incubation for 1 h at 37 °C and four washings with PBS–Tween, 100 μL goat anti-rabbit IgG peroxidase conjugate (1/500 in gelatin–PBS) was added to each well and incubated for 30 min at 37 °C. Plates were then washed 8 times with PBS–Tween, and bound peroxidase activity was determined as described earlier. Chicken IgG was used to prepare a standard curve in each plate, and results were expressed as nanograms of IgG per gram of turkey roll.

Statistics. Basic statistics and one-way analysis of variance were performed using MSTAT software (MSTAT, 1989). Mean separations were performed using Tukey's test with the mean square error term at the 5% level of probability.

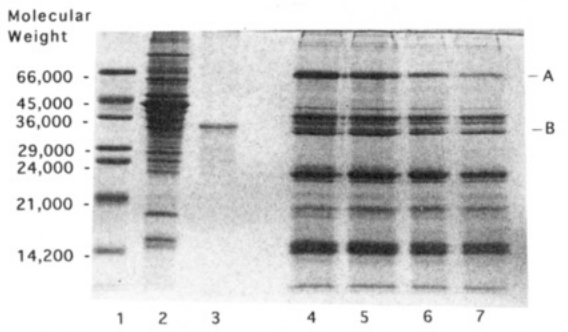

Figure 1. Representative sodium dodecyl sulfate–polyacrylamide gel electrophoretogram of muscle extracts from turkey rolls heated to different endpoint temperatures: (A) turkey serum albumin (TSA); (B) lactate dehydrogenase (LDH); (lane 1) molecular weight marker; (lane 2) raw turkey roll; (lane 3) chicken muscle LDH (from Sigma); (lane 4) 68.9 °C; (lane 5) 70.0 °C; (lane 6) 71.1 °C; (lane 7) 72.2 °C. Twenty micrograms of protein was loaded on each gel lane for turkey rolls.

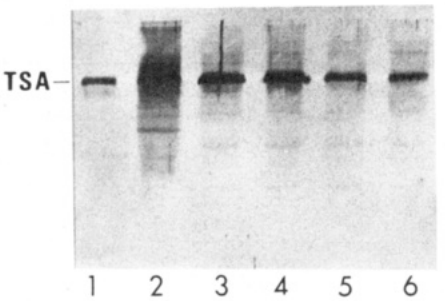

Figure 2. Western blot of sodium dodecyl sulfate–polyacrylamide gel of TSA and muscle extracts from turkey thigh rolls heated to different endpoint temperatures: (lane 1) TSA; (lane 2) raw turkey roll; (lane 3) 68.9 °C; (lane 4) 70.0 °C; (lane 5) 71.1 °C; (lane 6) 72.2 °C. The amounts of protein loaded on the gel lane were 0.1 and 10 μg for TSA and turkey rolls, respectively. Polyclonal antibodies to turkey serum proteins (PAb-TS) were used.

RESULTS AND DISCUSSION

Identification of Protein Indicators. Two protein bands (A and B) in meat extracts decreased in intensity when the processing temperature of turkey thigh rolls was increased (Figure 1). LDH (band B) was identified on the basis of the work of Wang et al. (1992). The molecular mass of the LDH subunit is 35 000 (Holbrook et al., 1975). SDS–PAGE of turkey thigh rolls (Figure 1) showed that a single LDH band was observed in cooked turkey thigh rolls (lanes 4–7). Similar band patterns were observed in meat processed to 68.9 and 70.0 °C (Figure 1, lanes 4 and 5).

The intensity of a 66 000-Da band (band A) decreased in meat processed to 71.1 °C (lane 6). A change in intensity of a 66 000-Da band was also found in turkey breast rolls processed from 68.3 to 72.1 °C (Wang et al., 1992) and turkey ham processed from 66.7 to 73.9 °C (Desrocher, 1994). Western blot analysis of SDS gels showed that PAb-TS reacted with TSA and turkey thigh roll extracts (Figure 2). A major band with the same mobility as TSA was observed in cooked turkey thigh rolls. These results indicated that the major band in cooked turkey thigh rolls detected by PAb-TS was TSA. Furthermore, the intensity of the TSA band identified by Western blotting decreased as the processing temperature of the turkey thigh rolls increased.

IgG is the second most abundant serum protein (Berne and Levy, 1988). Western blot analysis of SDS gels revealed that PAb–IgG reacted with chicken IgG and raw turkey thigh roll extracts, but only faint bands were observed in cooked meat extracts. Desrocher (1994) made PAbs against TSA and identified a 66 000-Da protein extracted from turkey ham as TSA. Thus, the 66 000-Da protein found in SDS–PAGE of cooked turkey thigh rolls was probably TSA.

Table 2. Influence of Processing Temperature on Extractable Protein Content and Lactate Dehydrogenase (LDH) Activity of Turkey Thigh Roll[a]

internal temp (°C)	extractable protein (%)	LDH activity (units/g of sample)
raw	18.3 ± 1.0[b]	274.5 ± 30.3[c]
68.9 ± 0.3	4.2 ± 0.4[c]	108.0 ± 21.2[d]
70.0 ± 0.3	3.8 ± 0.3[cd]	81.6 ± 7.2[de]
71.1 ± 0.3	3.3 ± 0.2[d]	70.2 ± 2.7[e]
72.2 ± 0.3	3.3 ± 0.3[d]	43.7 ± 7.8[f]

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

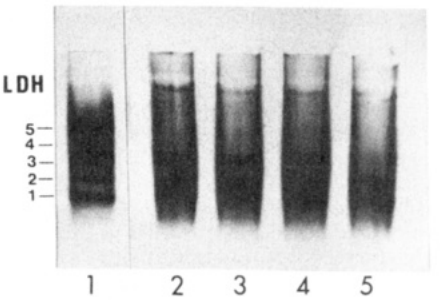

Figure 3. Representative native polyacrylamide gel electrophoretogram with LDH-specific stain of muscle extracts from turkey thigh rolls heated to different endpoint temperatures: (lane 1) raw turkey roll; (lane 2) 68.9 °C; (lane 3) 70.0 °C; (lane 4) 71.1 °C; (lane 5) 72.2 °C. Ten and twenty micrograms of protein were loaded for raw and heated samples, respectively.

LDH as Endpoint Indicator. Extractable protein decreased from 18.3% in raw turkey rolls to 4.2% at an internal processing temperature of 68.9 °C (Table 2). A decrease ($P < 0.05$) in extractable protein between 68.9 and 71.1 °C was observed. However, extractable protein content did not differ ($P > 0.05$) in turkey thigh rolls processed to 70.0 °C and above. LDH activity differed ($P > 0.05$) in rolls processed to 68.9 and 71.1 °C. LDH activity did not differ in turkey rolls processed to 70.0 and 71.1 °C; however, a decrease ($P < 0.05$) in LDH activity was observed between 71.1 and 72.2 °C.

Five turkey thigh LDH isozymes were observed on polyacrylamide gels stained with LDH specific stain (Figure 3, lane 1). LDH is a tetrameric molecule. The five isozymes consist of five different combinations of two different polypeptide chains called H and M; the electrophoretic labels LDH-1, -2, -3, -4, and -5 refer to the H_4, H_3M, H_2M_2, HM_3, and M_4 tetramers, respectively (Holbrook et al., 1975). Only LDH-1, LDH-2, and LDH-3 were observed in turkey thigh rolls processed to 68.9, 70.0, and 71.1 °C (Figure 3, lanes 2–4). These results indicated that LDH-1, LDH-2, and LDH-3 were more heat stable than LDH-4 and LDH-5. LDH-3 was not found in turkey thigh rolls processed to 72.2 °C (Figure 3, lane 5).

The LDH activity was 70.2 and 43.7 units/g in extracted turkey thigh rolls processed to 71.1 and 72.2 °C, respectively. However, less than 2 units/g of LDH activity remained in turkey breast rolls processed to the same temperatures (Wang et al., 1992, 1993). Wang et al. (1992) showed that LDH-5 was the predominant isozyme in turkey breast rolls. This isozyme was not detected when thigh rolls were heated to 68.9 °C and above. Thus, the higher LDH activity in cooked turkey thigh rolls was attributed to the presence of more heat-stable isozymes in thigh muscle.

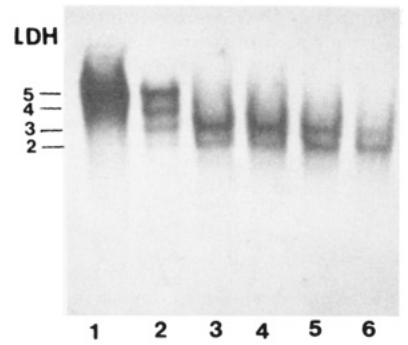

Figure 4. Western blot using polyclonal antibodies of native gel of turkey breast muscle LDH and muscle extracts from turkey thigh rolls heated to different endpoint temperatures: (lane 1) isolated turkey breast muscle LDH; (lane 2) raw turkey roll, (lane 3) 68.9 °C; (lane 4) 70.0 °C; (lane 5) 71.1 °C; (lane 6) 72.2 °C. Protein amounts loaded on the gel lanes were 0.2, 2.5, and 20 μg for isolated turkey muscle LDH, raw turkey roll, and heated turkey rolls, respectively.

Table 3. Influence of Processing Temperature on Lactate Dehydrogenase (LDH) Content in Turkey Thigh Rolls As Measured by Sandwich Enzyme-Linked Immunosorbent Assay (ELISA)[a]

internal temp (°C) of turkey rolls	LDH content (ng/g of sample)
raw	217124 ± 46778[b]
68.9 ± 0.3	461 ± 60[c]
70.0 ± 0.3	346 ± 38[cd]
71.1 ± 0.3	305 ± 51[d]
72.2 ± 0.3	146 ± 45[e]

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

Western blot of native polyacrylamide gels of turkey thigh roll extracts with polyclonal antibodies (Figure 4) showed that there were three major bands (LDH-3, -4, and -5) and a faint band (LDH-2) in raw turkey thigh rolls (lane 2). These results indicated that polyclonal antibodies raised against turkey muscle LDH only recognized isozymes containing the M form. The intensities of LDH-3 and LDH-2 bands were similar at processing temperatures of 68.9, 70.0, and 71.1 °C (lanes 3–5), whereas a less intense LDH-3 band was observed at 72.2 °C. An LDH-3 band was detected at 72.2 °C in a Western blot, but it was not observed on polyacrylamide gels stained to detect enzyme activity. Some LDH-3 molecules were probably partially denatured, which caused a conformational change in the active site region and loss of enzyme activity, but such change did not influence the binding of antibodies to another portion of the LDH-3 molecule.

The LDH content measured by sandwich ELISA for raw turkey thigh rolls was 217 120 ng (Table 3), which was about $^1/_{10}$ of that in turkey breast rolls (Wang et al., 1993). The LDH contents of thigh rolls processed to 70.0 and 71.1 °C did not differ ($P > 0.05$). Two major bands (LDH-4 and LDH-5) and one faint band (LDH-3) were detected on Western blots (Figure 5, lane 2) of native polyacrylamide gel containing raw turkey thigh roll extracts with LDH MAb D5E. These results suggested that MAb D5E mainly bound LDH isozymes with at least two subunits of M form. Thus, the lower LDH content of raw turkey thigh rolls as compared to breast rolls was due to the differential binding ability of MAb D5E to LDH isozymes.

Changes in LDH activity, LDH content, and LDH-3 intensity were found between turkey thigh rolls processed to 71.1 and 72.2 °C. Results suggested that these assays could differentiate turkey thigh rolls processed

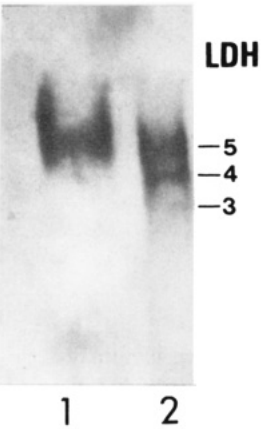

Figure 5. Western blot using monoclonal antibody of native gel of isolated turkey breast muscle LDH and muscle extract from raw turkey thigh rolls: (lane 1) isolated turkey breast muscle LDH; (lane 2) raw turkey roll. Protein amounts loaded on the gel lanes were 0.4 and 5 μg for isolated turkey muscle LDH and raw turkey roll, respectively.

Table 4. Influence of Processing Temperature on the Content of Serum Proteins and Immunoglobulin G (IgG) in Turkey Thigh Rolls As Measured by Enzyme-Linked Immunosorbent Assay (ELISA)[a]

internal temp (°C) of turkey rolls	turkey serum protein content[b] (mg/g sample)	IgG content[c] (ng/g sample)
raw	39.22 ± 13.43[d]	658243 ± 289102[d]
68.9 ± 0.3	2.36 ± 0.37[e]	1258 ± 389[e]
70.0 ± 0.3	1.82 ± 0.30[ef]	1097 ± 193[e]
71.1 ± 0.3	1.23 ± 0.27[fg]	771 ± 287[e]
72.2 ± 0.3	0.89 ± 0.23[g]	605 ± 254[e]

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

to these temperatures. Moreover, turkey thigh rolls are often cooked commercially to a minimum internal temperature of 73.9 °C (165 °F) to extend shelf life (Weiner, 1987; Weiner, personal communication, 1992) and to ensure safety in products cooked in food service establishments (FDA, 1993). Because of the higher stability of thigh LDH isozymes, LDH activity and LDH ELISAs were better suited for detection of higher endpoint cooking temperatures in products containing thigh meat.

Serum Proteins as Endpoint Indicators. A sandwich ELISA with PAb-TS as capture antibodies and biotin-labeled PAb-TS as detection antibodies was developed to detect turkey serum protein in meat products. Turkey serum protein content decreased as the internal processing temperature of turkey thigh rolls was increased (Table 4). A decrease ($P > 0.05$) in turkey serum protein content was found between 68.9 and 71.1 °C and between 70.0 and 72.2 °C. There was no difference ($P > 0.05$) in serum protein content between turkey rolls processed to 70.0 and 71.1 °C. These results suggested that serum protein ELISA could differentiate turkey thigh rolls processed to 68.9 and 71.1 °C. The IgG content in cooked meat extracts determined by CI-ELISA was low and did not differ in rolls processed between 68.9 and 72.2 °C (Table 4). This was consistent with Western blot analysis described earlier.

Conclusion. The LDH activity in cooked turkey thigh rolls was higher than that in breast rolls due to heat-stable isozymes in thigh muscle. The LDH ELISA could not be used to determine the endpoint temperature of turkey thigh products processed between 68.9 and 71.1 °C due to the presence of heat-stable LDH

isozymes in thigh muscle. LDH ELISA might be better suited to differentiate turkey thigh rolls processed to temperatures above 71.1 °C. The decrease in the intensity of a 66 000-Da protein was attributed to the loss of TSA during thermal processing of turkey thigh rolls. Serum protein ELISA could be used to differentiate turkey thigh rolls processed between 68.9 and 71.1 °C. TSA was the major serum protein remaining in cooked turkey thigh rolls. Only small quantities of IgG remained in turkey thigh rolls processed to 68.9 °C and above.

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