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INVESTIGATION OF THE USE OF ELISA AS A RAPID ASSAY INDICATOR FOR VERIFICATION OF COOKING TEMPERATURE ENDPOINT FOR MEAT PRODUCTS

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INTRODUCTION

In the United States, there are over 12.5 million cases of foodborne disease each year that cost the U.S. over $8 billion annually (Todd, 1989). In response to this issue, current recommendations by the scientific community include the development of verification procedures that would better ensure the safety of food products (CAST, 1994). The U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) mandates minimum final cooking temperatures for various precooked meat products to ensure the safety of these products (USDA, 1993). Current methods of verifying minimum cooking temperatures in beef and pork products include the residual acid phosphatase assay (USDA, 1986a), coagulation test (USDA, 1986b), and catalase test (USDA, 1989). However, present methods of assessing cooking endpoint temperatures have been shown to be subjective, variable, and lacking accuracy for predicting the actual heating endpoint achieved during cooking (Townsend et al., 1984, Townsend, 1989).

Collins et al. (1991a,b) identified bovine lactate dehydrogenase (LDH) as a potential endpoint indicator. Wang et al. (1992) proposed the use of LDH in an enzyme-linked immunosorbent assay (ELISA) as a test to determine the endpoint cooking temperatures in processed turkey rolls. The ELISA test was capable of determining minimum cooking temperature to within 1.1°-1.2°C in the temperature range of 68.3°-72.1°C. This experiment was designed to investigate if a similar test could be developed for red meats.

MATERIALS AND METHODS

Pork hearts were obtained from commercially slaughtered hogs within 48 h postmortem. Cores (30 g) were removed and placed in 50 ml, flat top cap disposable sterile centrifuge tubes (Corning Glassworks, Corning, NY 14831) and cooked in a water bath to raw, 60°, 65°, 70°, 75°, 80°, 85°, 90°, 95°, and 100°C internal temperature in water set at 2° to 3°C above the final temperature endpoint. Internal temperatures were monitored by a digital thermometer (Omega HH82 digital thermometer, Omega Engineering, Stamford, CT) with a thermocouple placed in the geometric center of each core.

Samples were extracted by the modified procedures of Wang et al. (1992) by homogenization of 25 g meat with 75 ml PBS in a Waring blender for 90 s (three repetitions of 30 s on, 10 s off). Homogenates were centrifuged at 16,000g for 20 min at 4°C and supernates poured off and saved. Protein concentrations were determined by the BCA method (Pierce Chemical Co., Rockford, IL 61105). Porcine lactate dehydrogenase isozyme 1 (LDH-1) in $(NH_4)_2SO_4$ were obtained (Sigma Chemical, St. Louis, MO 63178) and dialyzed (Pierce Biotec Co., Rockford, IL 61105) in ddH_2O.

To determine protein content and antigenicity of the meat extracts, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separations were performed using a Mini-Protean II electrophoresis unit (Bio-Rad Laboratories, Richmond, CA 94547) and a power supply (Bio-Rad Model 1000/500). Stacking and separating gels were 3 and 10% acrylamide, respectively. Molecular weights were determined on gels by comparing relative mobilities of protein bands to those of molecular weight standards (Bio-Rad SDS-PAGE broad range prestained and unstained standards). Protein bands were stained with Coomassie Brilliant Blue stain. Porcine LDH-1 standard was used to identify LDH-1 bands in meat extracts.

Gels were transferred for Western Blot Analysis electrophoretically (12 h at 10 V then 1 h at 25 V) from SDS-PAGE gels to polyvinylidene difluoride (PVDF) membrane in a Mini Trans-Blot unit (Bio-Rad) in transfer buffer, then blocked in 5% nonfat dry milk (NFDM) in TTBS (pH 7.5 TBS with .05% Tween 20) for 1 h at 37°C. Then the membrane was transferred to the primary antibody. A rabbit produced porcine LDH-1 polyclonal antibody was purchased (Hycor Biomedical, Irvine, CA, 92715) and used as a primary antibody. Primary antibody transfer was completed in a 1:100 dilution in 1% NFDM in TTBS for 2 h at 37°C. Then, membrane was washed in 3X TTBS, 5 min per wash on a shaker (Orbit Shaker, Lab Line), transferred to a secondary antibody (1:1000 dilution of anti-rabbit conjugated horseradish peroxidase in 1% NFDM), and incubated for 1 h at 37°C. Membrane then was washed again 3X in TTBS, 5 min per wash. Bound peroxidase was determined by covering with 50 ml of substrate solution (45 ml 50 mM Tris [pH 7.6], 30 mg 3,3' diaminobenzidine tetrachloride, 5 ml 0.3% $NiCl_2$, and 50 µl

H_2O_2) for 15 min, washed in 50 ml ddH_2O for 5 min and air dried on a paper towel. Visible bands of antibody reaction indicated antigenicity of LDH-1 in samples.

RESULTS

Absence of antigenic bands demonstrated loss of porcine LDH-1 solubility between 70 and 75°C on Coomassie Blue stained SDS gels. Loss of protein solubility of LDH-1 corresponded to a loss of antigenicity of LDH-1 as observed with the Western Blot ELISA. Total protein contents of the meat extracts showed a significant reduction in total protein content as samples were heated from 4° to 100°C.

These results indicate that porcine LDH-1 could serve as a potential cooked temperature indicator to rapidly verify that pork products had been heated to a minimum final endpoint temperature of between 70 and 75°C.

Results of this study provide information on the potential for porcine LDH and other proteins as a safe cooking point indicator when adapted to rapid immunological assays suitable for pork products. Should similar results be observed with skeletal muscle tissue, a rapid assay for determining the previous heat treatment of pork roasts or other products could be developed. Further study using these methods may lead to the discovery of proteins that could be used to determine heating endpoints in other meat products.

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