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MONOCLONAL ANTIBODY DEVELOPMENT AND SANDWICH ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE PROTEIN MARKER LACTATE DEHYROGENASE TO DETERMINE ENDPOINT TEMPERATURES OF GROUND BEEF

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INTRODUCTION

Most outbreaks of *Escherichia coli* O157:H7 have been associated with consumption of undercooked ground beef products. The severity of symptoms, including death in some cases, have made control of this organism a concern for meat processors, government regulatory agencies, food retailers and consumers. Studies of thermal inactivation in ground beef have shown that *E.coli* O157:H7 is heat sensitive (Line et al., 1991). Hence, proper cooking is one simple method to eliminate the pathogen from food. The meat industry is recommending that hamburger patties be cooked to a minimum temperature of 69.4°C and held for 10 sec.

To verify proper cooking of beef products, the U.S. Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) currently employs a protein coagulation test (USDA, 1986) or a catalase enzyme test (USDA, 1989). These methods are considered unreliable and do not provide an adequate margin of safety. Lactate dehydrogenase (LDH) has been shown to be a potential endpoint temperature (EPT) indicator in turkey products (Wang et al., 1992; Abouzied et al., 1993; Wang et al., 1993), beef model systems (Collins et al., 1991; Stalder et al., 1991) and hamburger patties (Smith et al., 1995). The quantification of an enzyme indicator by enzyme-linked immunosorbent assay (ELISA) has been successfully used for the determination of EPT in poultry products (Wang et al., 1992; Abouzied et al., 1993; Wang et al, 1993).

The goal of this project was to develop an accurate immunoassay to verify proper heat processing of ground beef patties. Monoclonal antibodies (MAb) to lactate dehydrogenase were prepared and a sandwich ELISA, using MAb as capture antibodies and polyclonal antibodies as detector antibodies, was developed. The sandwich ELISA was verified at different processing temperatures in model systems and commercial products. The effect of fat content and freeze-thaw cycles on LDH concentration was determined.

METHODS

Production of MAb. Two groups of 6-8 week-old female BALB\c mice were injected either subcutaneously or intraperitoneally (five mice each) with 75 μg of bovine muscle LDH. MAb were produced following the procedure of Galfre and Milstein (1981) as modified by Abouzied et al. (1990).

Indirect and sandwich ELISA. An indirect ELISA was performed as described by Abouzied et al. (1990) and used to determine antibody titer. A competitive indirect ELISA was used to test cells for antibody production and was identical to the indirect ELISA, except that after blocking of non-specific sites and washing, 50 μL of LDH were added to each well with 50 μL of LDH antisera or 50 μL of cell culture supernatant. The sandwich ELISA was performed as described by Orta-Ramirez (1994).

Model system. Ground beef (from chuck) or eye of round (semitendinosus muscle) was purchased from a local store. Moisture, fat and protein content were determined according to AOAC (1990) methods 950.46B, 991.36 and 981.10, respectively.

Ground beef (2.5 g) was placed in 10 x 75 mm glass thermal death time (TDT) tubes. The tubes were sealed with teflon tape and heated to internal temperatures between 62 to 74°C at 2°C intervals in a circulating water bath connected to a temperature programmer. The temperature of the water bath was set 0.5°C above the target temperature. Internal temperature was monitored with a thermocouple inserted in the center of a control TDT tube containing 2.5 g meat. Tubes were removed when they reached the target temperature and immediately placed in an ice-water bath.

LDH was extracted using 3 volumes (w/v) of 0.15 M NaCl, 0.01 M Na phosphate buffer, pH 7.2. The sample was vortexed for 30 sec and then shaken 20 min at 4°C. The extract was centrifuged at 5,000 x g for 10 min at 4°C. The supernatant was collected after filtration through Whatman No. 1 filter paper. The LDH concentration of the extracts was determined by sandwich ELISA.

In another experiment, fat content of ground semitendinosus muscle was adjusted to 10, 15 and 20 % using ground beef kidney fat. Raw meat extracts and extracts of meat cooked to 69.4°C as previously described were analyzed by sandwich ELISA. The effect of four freeze-thaw cycles on the concentration of LDH in raw ground beef and ground beef cooked to 69.4°C was determined.

Commercial patties. Three groups of hamburger patties cooked to 54.6-65.6, 68.7-71.1, or 73.9-83.2°C were obtained from a commercial processor. Ten grams of meat from the center of each patty was extracted and analyzed as described above by sandwich ELISA. Four patties were analyzed from each temperature range.

Statistical analysis. Each experiment was performed in triplicate. 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.

RESULTS AND DISCUSSION

Production of MAb and ELISA development. Mice injected subcutaneously with bovine muscle LDH had higher titers than those injected intraperitoneally. In addition, when tested by competitive indirect ELISA, sera of mice injected subcutaneously showed

higher inhibition then those injected intraperitoneally. A total of 360 wells were seeded with fused cells from the spleen of the mouse with the highest titer and NS-1 myeloma cells, but only one produced antibodies against LDH. A second fusion was performed using two additional mice which yielded two more positive hybridomas. The three antibody-producing hybridomas, designated as 5E2, 4C5 and 5F8, were further expanded and cloned. Eight stabilized lines were obtained which showed high inhibition when tested by indirect competitive ELISA. A sandwich ELISA was developed with MAb as the capture antibodies and polyclonal antibodies as the detector antibodies. Minimum detection limit was 1 ng LDH/ml extract. No cross-reactivity was observed with the antibodies used in the sandwich ELISA and LDH from bovine heart, porcine heart, rabbit muscle or chicken muscle. Slight cross-reactivity was observed with porcine muscle at concentrations of 2 ng LDH/ml extract or above.

Detection of LDH. The ground beef used in the model system contained 63.3±0.18% moisture, 19.6±0.94% fat and 16.7±0.45% protein. The LDH content of extracts decreased from 979.8 μg/g meat in ground beef cooked to 62°C to 0.24 μg/meat in beef cooked to 74°C (Table 1). LDH content of commercially cooked patties also decreased as the processing temperature was increased (Table 2). Similar concentrations of LDH (about 3 μg/g meat) were observed in both the model system and commercially cooked patties at a cooking temperature of 70°C. LDH concentration of raw ground beef decreased as fat content was increased, but no differences in LDH concentration were observed when ground beef was heated to 69.4°C.

CONCLUSIONS

The LDH content of ground beef decreased as cooking temperature was increased. Results in both the model system and commercial patties suggest that a maximum concentration of about 3 μg LDH/g meat might indicate that ground beef was processed to 70°C or above. For routine use, the ELISA can be prepared as a rapid self-contained field assay that can be performed in 30 min or less.

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Table 1. Lactate dehydrogenase (LDH) content of cooked ground beef as measured by sandwich ELISA

Temperature (C)	LDH (μg/g meat)
62	979.82±45.57
64	804.92±17.43
66	589.84±62.38
68	122.03±18.42
70	3.03±0.54
72	0.43±0.15
74	0.24±0.01

Table 2. Lactate dehydrogenase content (LDH) of cooked commercial beef patties as measured by sandwich ELISA

Temperature (C)	LDH (μg/g meat)
54.4-65.6	362.44±179.88
68.3-71.1	3.38±2.78
73.9-82.2	0.30±0.15