GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) ANALYSES AS DETECTORS OF CENTRAL NERVOUS SYSTEM TISSUE IN MEAT PRODUCTS

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Background

The presence of brain or spinal cord material as an inadvertent contaminant of meat may result from stunning livestock, splitting the carcass, or preparing advanced meat recovery (AMR) products from the vertebral column (FSIS/USDA, 1998). In light of current consumer concern about bovine spongiform encephalitis (BSE), a disease transmitted by consumption of central nervous system (CNS) tissue, a reliable analytical test for CNS tissue in meat products is essential to ensure consumer confidence and allay consumer fears of BSE in meat products.

The difficulty of observing CNS in meat products led us to seek more sensitive methods to detect CNS contamination. The current methods to detect CNS tissue in blood, lungs, or meat include gross tissue dissection and visual examination (Bauer et al., 1996; Garland et al., 1996), analysis of cholesterol (Lücker & Bülte, 1998), or histological preparation and microscopic examination of samples (Kelly, Hafner, McCaskey, Sutton & Langheinrich, 2000). These methods are cumbersome, insensitive, time consuming and costly. This prevents the examination of many samples to evaluate cattle-stunning methods or preparation techniques for AMR product (FSIS/USDA, 1998). Other methods for the detection of CNS tissue have been reported more recently. British scientists detected the presynaptic protein, syntaxin 1B in jugular venous blood obtained after stunning cattle and sheep with captive bolt guns (Anil, et al., 1999; Love, et al., 2000; Anil, et al. 2001). Lücker, Egenbrodt, Wenisch, Leiser & Bülte (2000), used quantification of cholesterol and immunochemical detection of neuron-specific enolase and glial fibrillary acidic protein (GFAP) to detect CNS tissue in heat-treated meat products. GFAP is the major membrane protein of glial filaments in differentiated astrocytes, which are restricted to the CNS (Eng & Lee, 1995). The antigenicity of GFAP has permitted the preparation of highly avid and specific polyclonal and monoclonal antibodies (Eng, Ghirniker and Lee, 2000). GFAP immunohistochemistry has been used for the diagnosis of astrocytic tumors, the study of astrocyte development and gliosis and the study of CNS regeneration and transplantation (Eng, et al., 2000).

Detailed protocols have been reported for quantifying mouse GFAP by a colorimetric microtiter plate-based sandwich enzyme-linked immunosorbant assay (ELISA) (O'Callaghan, 1991). We demonstrated that the GFAP ELISA provided a valid, sensitive and repeatable method to detect CNS tissue contamination in beef meat and that the antigen is relatively stable when stored at 4°C (Schmidt et al., 1999). In a recent study (Schmidt et al., 2001), a fluorescent ELISA for GFAP with increased sensitivity to detect CNS tissue in meat products was reported (GFAP F-ELISA). The fluorescent assay was sensitive to 0.2 ng GFAP, had an intra-assay coefficient of variation (CV) of 2.0% and an inter-assay CV of 14.1%. Bovine spinal cord and brain demonstrated dose-response curves which were parallel to GFAP standards while peripheral sciatic nerve and cervical ganglia also cross-reacted at high tissue levels. The use of another CNS marker, Syntaxin 1-B, was not effective for neural tissue detection when meat products were sampled. Less than 1.0 ng GFAP/mg tissue was found on most beef subprimals and AMR product. Occasional samples contained higher levels of GFAP, probably due to contamination by the carcass splitting saw, incomplete removal of the spinal cord or a chance sampling of nerve. Further reduction of CNS content was achieved by removal of the cervical vertebrae and the spinal canal prior to processing beef chuck bones through AMR equipment. The presence of GFAP was very low (0.037 ng/mg) in beef patties collected from major processors throughout the USA.

Another study (Schmidt *et al.*, 2002) independently validated the results of a test kit (Ridascreen® Risk Material 10/5 GFAP test kit, R-Biopharm, Inc., Darmstadt, Germany) with the GFAP F-ELISA. Both a swab and a homogenization method were used to prepare meat samples for GFAP kit analysis. Levels of GFAP in chuck, ground chuck and AMR were assessed with three methods (kit swab, kit homogenate, F-ELISA). GFAP concentrations were measured by the three methods and simple correlations were calculated. All correlations were greater than 0.9 and indicated the methods were capable of detecting spinal cord in a dose-responsive manner in ground beef.

Objective: The effects of heating meat products containing known amounts of neural tissue was examined to determine if GFAP could be detected in cooked meat products in the presence and absence of sausage additives.

Methods: Approximately 9 kg of AMR, 9 kg beef chuck shoulder clod and 200 g spinal cord were obtained 48 hours post mortem from fed cattle. The beef chuck shoulder clod (3.5 kg) and the AMR (3.5 kg) were separately chopped in a six blade, 35 l bowl chopper (Meissner/RMF, Kansas City, MO) at 4,000 rpm for 15 revolutions of the bowl. A second 3.5 kg batch of each meat source was chopped similarly with typical sausage ingredients according to the following formulation: 78.96% meat; 15% water; 1.6% salt; 0.15% alkaline phosphate; 0.20% cure salt (6.25% sodium nitrite); 0.04% sodium erythorbate; 2.0% dextrose; 1.0% ground mustard; 0.1% coriander, 0.2% nutmeg; 0.1% cardamom, 0.35% paprika; 0.3% ground black pepper. Spinal cord was chopped for 30 sec in a food processor. Portions (500 g) of each of the 4 treatments were mixed in a Kitchen Aid mixer at high speed for 30 sec with 0, 0.2, 0.4, 0.6, 0.8 or 1.0% chopped spinal cord. Six 60 g samples were removed from the mixtures, individually vacuum packaged and cooked for 1 hr in an agitated water cooker at either 60, 66, 71, 77, 82°C or left raw. Samples were chilled in ice water and stored at 4°C until extracted for analyses of GFAP as described by Schmidt *et al.* (2001).

Results: Figures 1 and 2 show the effect of heating and sausage spices on the detection of GFAP in meat containing known amounts of added spinal cord. Regardless whether the product was raw or heated to 60-82°C for 1 hr., the spinal cord was detectable in product manufactured from beef chuck clod (Fig. 1) or beef AMR (Fig. 2). The presence of spices and other sausage ingredients reduced apparent GFAP concentration. The reduction was the result of lower meat content in the sausage and less water release on cooking from the spice/salt/phosphate/water treatment which had a cook yield of 99% versus a cook yield of 75% for the non-spice treatments. The variation at a given level of spinal cord was probably due to the inability to uniformly homogenize the spinal cord into the mix.

Figure 1. GFAP levels detected in raw or cooked (60-82°C for 1 hr) ground chuck and sausage with 0-1% added spinal cord

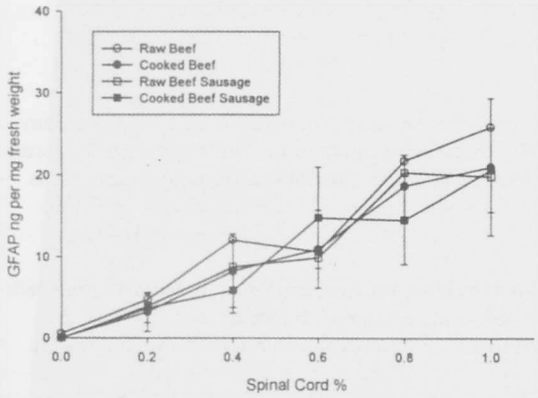
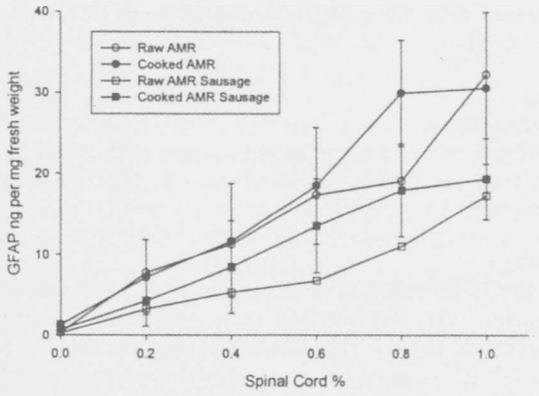


Figure 2. GFAP levels detected in raw or cooked (60-82°C for 1 hr) AMR product and AMR sausages with 0-1% added spinal cord



Pertinent Literature

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