
CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EVALUATION OF MORPHOLOGIC METHOD FOR THE DETECTION OF NERVOUS TISSUE IN MINCED MEAT

Javad Sadeghinezhad[1], Narges Zadsar[1], Zohreh Zohari[2], Kamand Fattahi[2], Bahador Hajimohammadi[2], Hassan Morovvati[1], Ali Khanjari[3], Roberto Chiocchetti[4*]

[1] Department of Basic Sciences, University of Tehran, Tehran, Iran;
[2]Research Center for Molecular Identification of Food Hazards, Shahid Sadoughi University of Medical Sciences, Yazd, Iran;
[3] Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran;
[4] Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy;
** roberto.chiocchetti@unibo.it*

Article history:
Received
15 March 2017
Accepted:
28 October 2017

Keywords:
Minced meat
Nervous tissue
Histology
Imuunohistochemistry

ABSTRACT

Producing meat products with ingredients which are not consistent with the label is considered fraud. Due to the high economic value of meat, the use of unauthorized tissue in meat products is possible. Aside from the adulteration aspect, it is important to note that some animal tissues like the brain and the spinal cord can bear infective agents which are transmissible to humans. Based on these observations, the aim of the present study was to apply morphological method for detection of nervous tissues in minced meat. Laboratory adulterated minced beef meat; each containing 0, 5, 10, 15 and 20% of beef brain was prepared. Then each sample was divided into three parts and four paraffin embedded blocks were prepared from each part. The sections were stained using sudan black and cresyl violet and also the immunohistochemical staining with fluorescent method were applied using anti-neurofilament 200 antibody for the determination of nervous tissue. Although the neuronal cell bodies and neuronal fibers were clearly detectable in Cresyl violet staining and sudan black staining, respectively, however, staining intensity did not show any difference according to different percentages of added brain. In contrary, immunohistochemical study revealed that neurofilament 200-immunolabeling was present in all percentages of added brain samples and the intensity of the labeling varying from weak to strong consisted by the increasing the amount of brain in samples.

In conclusion, the immunohistochemical technique with fluorescent method is an effective method for evaluations of additive brain tissue in minced meat with high sensitivity.

1. Introduction

Meat products may contain ingredients which are not consistent with the label (Ballin, 2010). Since only muscle tissue is considered as meat from the point of view of labeling (Gout et al., 2004), several reports are available for the detection of different animal tissues in the meat products, in which histological technique has been used (Tremlova & Starha 2003; Gout et al. 2004; Prayson et al. 2008a; Prayson et al. 2008b, Ghisleni et al., 2010; Botka-Petrak et al., 2011; Latorre et al., 2015; Sadeghinezhad et al., 2015).

Some additives in meat products are not only important in meat product quality but also for the food safety. The central nervous system

tissues (CNST) including brain and the spinal cord can bear infectious agents, such as prions can cause transmissible spongiform encephalopathies (Prusiner, 1998). There is increasing evidence that bovine spongiform encephalopathy (BSE) can be transmissible to humans as variant Creutzfeldt Jacob disease (Almond and Pattison, 1997; Ridley and Baker, 1999; Roma and Prayson, 2005). Therefore, various methods like direct tissue examination (Bauer et al., 1996) and cholesterol content analysis (Lucker et al., 1999; Schmidt et al., 1999) has been used for the detection of CNST in meat products. The standard histological method has been applied for examination of CNST in meat products with some contradictions in literature (Linke, 1959; Wnisch et al., 1999). However, immunohistochemical method (IHC) has been used for the detec¬tion of CNST in meat products due to high specificity (Wenisch et al., 1999; Tersteeg et al., 2002).

The current experimental study was designed to evaluate the neurohistological staining (sudan black and Cresyl violet) and immunohistochemical staining (using neurofilament antibody) for the determination of CNST in raw minced meat with known levels of added brain tissue.

2. Materials and methods

Preparation of meat samples- Samples of minced beef meat, each containing 0%, 5%, 10%, 15% and 20% beef brain were prepared. All the samples with different percentages were evaluated for flavour, tenderness, juiciness, and overall acceptability by 10 different people. Then, each sample was divided into three equal parts, four pieces of each part were collected, and fixed in 10% neutral-buffered formalin.

Histological study- The tissues were routinely processed for light microscopy and embedded in paraffin. Each paraffin-embedded block was cut into 7µm sections and mounted on polysined slides. Then, one slide from each block was taken and stained using Sudan black and Cresyl violet in order to detect the neurons and myelin sheats, respectively.

Immunohistochemical study- The mounted slides were dewaxed in xylene and then rehydrated through graded ethanol up to water. For antigen unmasking, sections were heated in sodium citrate buffer (pH 6.0) in a microwave (two cycles of 5 min at 800 W). To reduce the background, the tissues were incubated in PBS containing 20% normal goat serum for 1 h at room temperature (RT). The tissues were then incubated overnight at 4°C in a humid chamber, in a primary antisera (anti-NF200 rabbit polyclonal antibody, 1: 200 - N4142, Sigma-Aldrich) diluted in a suitable medium (1.8% NaCl in 0.01 M phosphate buffer containing 0.1% Na-azide). After washing in PBS (3×10 min), the tissues were incubated for 1 h at RT in a humid chamber in a secondary antibody (Goat anti-rabbit IgG FITC, 1:200 - Calbiochem-Novabiochem) diluted in PBS. The tissues were then washed in PBS (3×10 min) and mounted in buffered glycerol pH 8.6.

Light and fluorescence microscopy- The slides were observed under a microscope (Nikon E600, Japan) equipped with electronic eyepiece (E-eye, MB-2250, Germany) and the Axiovision software (Carl Zeiss, Oberkochen, Germany) for the detection of brain tissue. The appropriate filter cubes to distinguish fluorescein isothiocyanate (FITC) fluorescence aid to locate CNST by the presence of a fluorophore that labelled the antigen. The images were processed using Adobe Photoshop CS (Adobe system, San Jose, CA).

3. Results and discussions

The organoleptic evaluation showed that added CNST was not detectable in the prepared minced meat, also at high percentages.

The striated skeletal muscle fibers were detectable using Sudan black and Cresyl violet staining; in all the examined slides, the multiple nuclei were displaced to the periphery of the muscle fibers. The neuronal cell bodies were clearly detectable in Cresyl violet staining, while were less observable in Sudan black staining, due to the blue-black color of the myelin sheet, (Figure 1). However, staining

intensity did not show any difference according to different percentages of added brain.

Immunohistochemical study revealed that NF200-immunolabeling was present in all percentages of added brain samples (Figure 2). The intensity of the labeling varying from weak to strong consisted by the increasing the amount of brain in samples.

There are reports indicating that BSE can be acquired by humans with consuming contaminated beef which lead to the loss of confidence in consumption of meat and meat products (Wenisch et al., 1999). The bovine skull including brain, spinal cord, eyes and tonsils has been considered as specified risk materials and thus have been banned as raw materials in processed meat products by European Union (Tersteeg et al., 2002). Thus of particular concern, various methods have been used for the detection of the brain tissue in meat products (Gout et al., 2004).

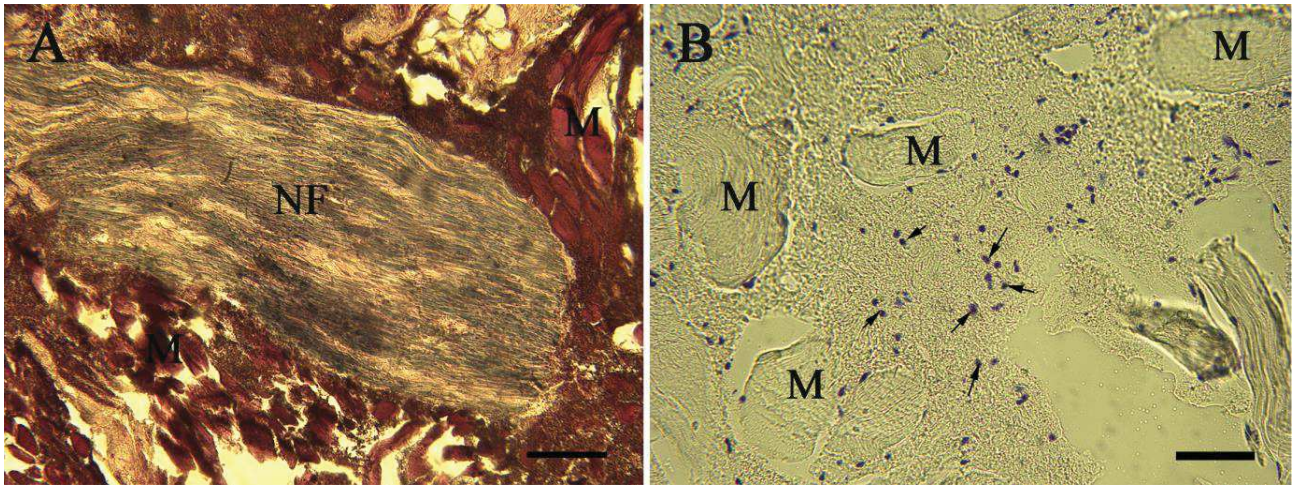

Figure 1. Photomicrograph of histological staining of additive nervous tissue in minced meat. A: The histological section shows the striated skeletal muscles (M). The Neuron fiber (NF) has been specified (Sudan black, scale bar 100 µm). B: The photomicrograph shows muscle fibers (M) and neuronal cell bodies (arrows) (Cresyl violet, scale bar 50 µm).

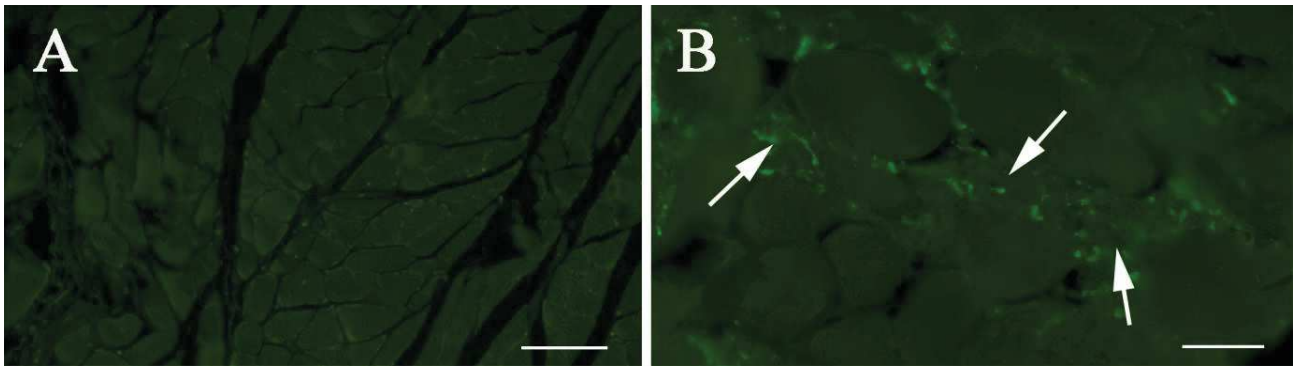

Figure 2. Photomicrograph of additive nervous tissue immunolabelling in minced meat with the antibody anti-NF200. A: In minced meat without nervous tissue (0 %) no reaction was seen (scale bar 100 µm). B: The neural fibers are easy recognizable (arrows) in minced meat with nervous tissue (scale bar 50 µm).

The histological technique used in this study despite revealed striated skeletal muscles and fragments of neuronal processes and the neuronal cells. Linke (1959) indicated the brain tissue in Frankfurter sausage using histological examination. In contrast, Wenisch et al. (1999), using H&E and also special stainings, stated that no neuronal cell body nor neuronal process were not detectable within cooked sausage due to homogenization in cutting mixer and heating pressure. In overall, this method cannot be recommended a reliable technique for the detection of CNST in minced meat because of destruction of the CNST in this product.

Immunohistochemical method (IHC) has mostly been used for the detec¬tion of CNST in meat products due to high specificity (Tersteeg et al., 2002). The neuron-specific enolase (NSE) immunoreaction was suggested as a reliable marker of CNST in cooked sausage as a consequence of the extraordinary resistance of the enzyme (Wenisch et al., 1999). However, Tersteeg et al. (2002) used four different antibodies including anti-neurofilament (NF), anti-myelin basic protein (MBP), anti-glial fibrillary acidic protein (GFAP) and NSE for detection of bovine (0%, 1%, 5%, 10%, and 20%) and porcine brain (5%) tissues in raw, pasteurized and sterilized meat products using DAB (3,3'-Diamino-Benzidine) staining. Overall, all the antibodies were useful for the raw meat product and the anti-MBP was suggested as a most useful antibody in detecting brain tissue of the heated meat products.

The problem with using IHC is the heating process or even certain meat manufacturing processes can cause conformational and chemical changes in the reactive side of the antigens (Tersteeg et al., 2002).

In minced meat despite the mechanical manipulation of the meat, the neuronal nuclei and processes are strongly labeled due to the lack of cooking in this type of product. Furthermore, florescent method for detection of the neuronal marker used in this study facilitate the detection of CNST and showed that staining intensity differ according to the different amount of added brain.

4. Conclusions

In conclusion, the immunohistochemical technique with fluorescent method is an effective method for evaluations of additive brain tissue in minced meat with high sensitivity. In addition, this method provide a facilitate detection of CNST in minced meat.

5. References

Ballin, N. Z. (2010). Authentication of meat and meat products. Meat Science. 86(3), 577–587.

Bauer, N. E., Garland, T., Edwards, J. F. (1996). Brain emboli in slaughtered cattle. Veterinary Pathology, 33, 600.

Botka-Petrak, K., Hraste, A., Lucic, H., Gottstein, Z., Gomerčić, M. D., Jaksic, S., Petrak, T. (2011). Histological and chem¬ical characteristics of mechanically deboned meat of broiler chickens. Veterinarski Arhiv. 81(2), 273–283.

Ghisleni, G., Stella, S., Radaelli, E., Mattiello, S., Scanziani, E. (2010). Qualitative evaluation of tortellini meat filling by histology and image analysis. International Journal of Food Science & Technology. 45(2), 265-270.

Gout, S., Valdivia, H., Hcdwell, D., Harris, N. (2004). Detection of neuronal tissue in meat using tissue specific DNA modifications. Biotechnologie, Agronomie, Société et Environnement. 8(4), 229-234.

Latorre, R., Sadeghinezhad, J., Hajimohammadi, B., Izadi, F., Sheibani, M. T. (2015). Application of Morphological Method for Detection of Unauthorized Tissues in Processed Meat Products. Journal of Food Quality and Hazards Control. 2, 71-74 .

Linke, H. (1959). Histologischer Nachweis von Ghirn in Bruh-wursten. Die Fleischwirtschaft. 57, 1766-1769.

Lucker, E., Eigenbrodt, E., Wenisch, S., Failing, K., Leiser, R., Bulte, M. (1999). Development of an integrated procedure for

the detection of of central nervous tissue in meat products using cholesterol and neuron-specific enolase as markers. Journal of Food Protection, 62(3), 268-276.

Prayson, B. E., McMahon, J. T., Prayson, R. A. (2008a). Applying morphologic techniques to evaluate hotdogs; what is in the hotdogs we eat? Annals of Diagnostic Pathology. 12(2), 98-102.

Prayson, B., McMahon, J. T., Prayson, R. A. (2008b). Fast food hamburgers: what are we really eating? Annals of Diagnostic Pathology. 12(6), 406-409.

Prusiner, S. B. (1998). Prions. Proceedings of the National Academy of Sciences USA. 95, 13363–13383.

Roma, A. A. Prayson, R. A. (2005). Bovine spongiform encephalopathy and variant Creutzfeldt-Jacob disease: how safe is eating beef? Cleveland Clinic Journal of Medicine. 72(3), 185-194.

Ridley, R.M., Baker, H. F. (1999). Big decisions on small numbers: lesson from BSE. Veterinary Quarterly, 21(3), 86-92.

Sadeghinezhad, J., Hajimohammadi, B., Izadi, F., Yarmahmoudi, F., Latorre, R. (2015). Evaluation of the Morphologic Method for the Detection of Animal and Herbal Content in Minced Meat. Czech Journal of Food Science. 33(6), 564–569.

Schmidt, G. R., Hossner, K. L., Yemm, R. S., Gould, D. H., O'Callaghan, J. P. (1999). An enzyme –linked immunosorbent assay for glial firillary acidic protein as an indicator of the presence of brain or spinal cord in meat. Journal of Food Protection. 62(4), 394-397.

Tremlova, B., Starha, P. (2003). Histometric evaluation of meat products: Determination of area and comparison of results obtained by histology and chemistry. Czech Journal of Food Sciences. 21(3), 101–106.

Tersteeg, M. H., Koolmees, P. A., van Knapen, F. (2002). Im-munohistochemical detection of brain tissue in heated meat products. Meat Science. 61(1), 67–72.

Wenisch,, S., Lücker, E., Eigenbrodt, E., Leiser, R., Bülte, M. (1999). Detection of central nervous tissue in meat prod¬ucts-An immunohistochemical approach. Nutrition Research. 19(8), 1165–1172.