WESTERN BLOT IMMUNOASSAY IN DETECTING MECHANICALLY DEBONED CHICKEN MEAT IN BEEF MIXTURES

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Abstract –Intentional substitution of mechanically deboned chicken meat (MDCM) in comminuted meat products has been a common fraudulent practice. The objective of this study was to detect MDCM in meat mixtures based on immunoreactions. Firstly, MDCM was added into beef at 0, 5, 10, 20 and 100% (wt/wt). Then, myosin binding protein C (MYBPC) antibody, as a specific protein for MDCM, was used in immunoblotting for detection of fraudulent substitution. According to the results of this study, MYPBC could be used in discriminating MDCM from beef at different ratios and this discrimination could be calculated even if with 5% addition of MDCM to the raw beef mixtures.

Key Words –Beef, Mechanically deboned chicken meat, Western blot assay

I. INTRODUCTION

Meat adulteration has been receiving great attention these days after the 2013 horse meat scandal in Europe. The most common adulteration applied in meat products in most countries is intentional substitution of valuable meats by low-cost ones for financial gain without informing consumers [1-2]. Mechanically deboned chicken meat (MDCM) is a by-product of poultry meat industry. This poor quality by-product is used in the formulation of low-cost comminuted meat products, sometimes as a source of fraudulent substitution. There are some analytical approaches for the detection of adulteration in meat products such as DNA-based (PCR, RT-PCR, etc.) [3], protein-based (electrophoretic, chromatographic, spectroscopic, etc.) [4], and immunological methods (ELISA, western blot, etc.) that have been used for authentication testing. Western blot immunoassay is a good candidate due to its high sensitivity and specificity to the species. Myosin binding protein C (MYBPC) is a muscle sarcoplasmic protein which has structural and regulatory roles. It has been identified as a potential MDCM biomarker in proteomics methods for the detection of MDCM adulteration in meat mixtures [5]. However, there is a lack of information regarding the use of MYBPC in detection of meat products, particularly in different ratios of substitution with immune-assay. Thus, the aim of this study was to investigate the use of western blot immunoassay in detecting fraudulent substitution of MDCM at various concentrations in raw beef mixtures.

II. MATERIALS AND METHODS

To prepare raw mixtures, MDCM was added into the minced beef from *Longissimus dorsi* muscles at the levels of 0, 5, 10, 20and 100% (wt/wt). Proteins were extracted according to Demiralp (2011) [6] Then, the separated protein bands in unstained SDS-polyacrylamide gels were transferred to PVDF (Polyvinylidene difluoride) membranes using Mini Trans-Blot® Electrophoretic Transfer Cell (Bio-Rad, USA). Blocking was performed by TBST containing 5% BSA g/ml for 1 hour at RT. Myosin binding protein C (MYBPC) antibody (ProteinTech, USA) for MDCM was used as a primer antibody which was diluted 1:2500 (ml/ml) in the antibody buffer (TBST containing 5% BSA [g/ml]) and incubated for 14 h at 4^{o}C. The incubation with diluted secondary antibody (1:20000) was performed with HRP conjugate secondary antibody (ProteinTech, USA) for1h. Finally, protein–antigens bands were visualized by chemiluminescence substrate Clarity™ Western ECL substrate (Bio-Rad, USA) using Odyssey® Fc Imaging System (LI-COR, Inc., USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Cell Signalling, USA) was used as housekeeping. The images were analyzed with the Image J software (NIH, USA).

III. RESULTS AND DISCUSSION

In the current study, both MYBPC and GAPDH proteins were detected in all samples with different band intensities (Figure 1). With the addition of MDCM, the band intensities of MYBPC showed a decrease when normalized by GAPDH. The intensity ratios shown in Figure 2 were calculated by dividing the intensities of

MYBPC bands to the intensities of GAPDH bands. The intensity ratio decreased with the increase in substitution of MDCM.

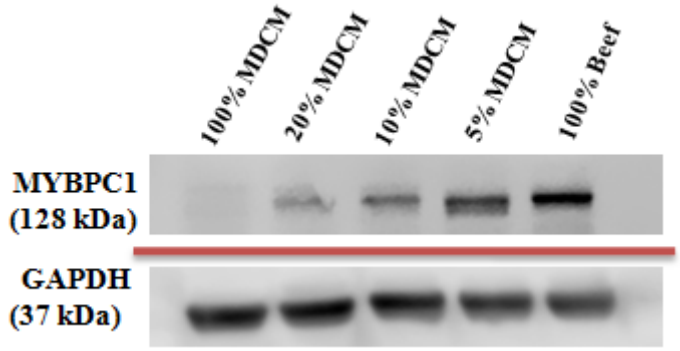

Figure 1. Western blot analysis of myosin binding protein C (MYBPC) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, for housekeeping) in extracts of beef-MDCM mixtures.

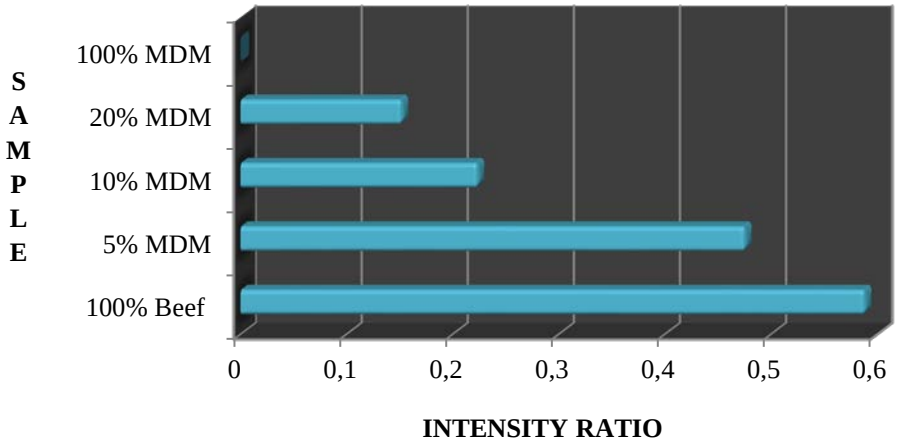

Figure 2. Intensities of myosin binding protein C (MYBPC) when normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH, for housekeeping) in the extracts of beef-MDCM mixtures

IV. CONCLUSION

Results from this study suggest western blot immunoassay with MYBPC could be used in detecting MDCM in beef mixtures. This method, suitable for screening purposes, provides a rapid detection of fraudulent mixtures and it could also be quantitative. With antibody-epitope interaction based sensors, detection of this kind contamination would be easier. These results show that MYBPC protein can be used for the detection of MDCM and differentiation of different amounts of MDCM contamination from the beef by western blot immunoassay.

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