DISTINGUISHING BETWEEN MSM AND NON-MSM MEAT: A MICROSCOPIC APPROACH

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I. INTRODUCTION

Mechanically separated meat (MSM) is derived from the meat left on animal carcasses once the main cuts have been removed. According to Regulation (EC) No 853/2004, MSM is referred to as "the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fibre structure" [1]. Based on the technology used to produce MSM, it is possible to distinguish between "low pressure MSM", and "high pressure MSM". Because of the tissue modification, MSM has an increased risk for microbial growth, due to nutrients release which provides a favourable substrate for bacterial growth. For this reason, it is compulsory to declare the presence and the percentage of MSM on the product label. Nevertheless, there is a suspicion that labelling requirements for MSM are not always fulfilled, because substitution frauds are reported with low-priced ingredients dishonestly used in the place of high-priced raw materials. The addiction of non-declared MSM in meat products not only decreases the quality of products themselves, but also misleads consumers and may involve associated health risks. In this regard, the BIOHAZ Panel of the European Food Safety Authority (EFSA) recommended the Organisms in charge of food inspections, such as veterinary services, reference laboratories, etc., to develop new approaches for the identification of MSM, because the currently used methods (i.e., rheological/textural parameters, dosage of protein, ash, cholesterol, iron, etc.), were judged as not fully reliable [2]. Among all tested parameters, only calcium content was significantly correlated with the presence of MSM in meat products, and a concentration of this element higher than 100 mg/100 g (1000 ppm) is attributed to the presence of MSM. Microscopy methods, alongside those which involve chemistry and molecular biology, represent yet another, sometimes less expensive, alternative for the examination and control of foods. Optical microscopy permits to identify all structures, by their morphological characteristics, and special stainings allow to highlight specific structures and chemical compounds, which colour differently from the other parts of the examined tissue [3]. This paper aims demonstrating that a simple light microscopy method can determine whether meat recovered from flesh-bearing bones of chicken could be distinguished from hand-deboned meat, based on techniques, pressure and machinery used.

II. MATERIALS AND METHODS

To develop a standardized microscopic protocol and to test its performance for MSM identification in poultry meat, reference samples were obtained by two plants authorized to produce MSM, located in northern Italy. In details, 20 reference high pressure MSM samples and 20 low pressure MSM samples were collected. In addition, 20 samples of freshly ground non-MSM chicken meat were collected, to test the specificity of the test. Histological preparation of samples involved that all samples were fixed in 10% neutral buffered formalin, automatically processed, paraffin-embedded, microtome cut into 3 ± 2 micrometre sections. Then, from each sample 2 histological preparations were obtained, for a total of 80 histological preparations used to test the sensitivity of the method: from each sample, one section was stained with haematoxylin-eosin, and one section was stained with *Von Kossa* stain (kit VON KOSSA method for Calcium 04 - 170801, Bioptica), to identify calcium ions (present in bone or

cartilage). Calcium salts are coloured in dark brown / black, while the remaining tissue assumes a very light pink nuance. Furthermore, a bovine lymph node with tuberculosis was used as a positive control. Two expert histopathologists independently examined, in blind evaluations, all samples by optical microscopy at increasing magnification, to classify them as MSM or non-MSM. Whenever the presence of bone or cartilage (highlighted by *Von Kossa* stain) were detected inside the tissue, samples were classified as MSM; otherwise, if only muscle, connective and adipose tissue were present, samples were considered as non-MSM. Results were then recorded into a spreadsheet. Accuracy, sensitivity, specificity, and inter-rater agreement with corresponding 95% confidence intervals (CI), were calculated. The inter-rater agreement was estimated by calculating kappa statistics. Statistical analysis was performed by using Stata/SE 14.0 (StataCorp, College Station, TX).

III. RESULTS AND DISCUSSION

All 80 MSM samples were correctly classified, because bone or cartilaginous tissues were identified among muscle tissue (predominant component), connective tissue and adipose tissue. Calcium compounds were present, with a dark brown colour, in all histological preparations containing MSM, regardless of it was produced at high or low pressure (Figure 1). Similarly, all non-MSM samples (N=20) were correctly recognised, because no calcium compound was highlighted with *Von Kossa* stain. Thus, by histological analysis it was possible to correctly distinguish all MSM samples from non-MSM samples. To distinguish between high and low pressure MSM samples, 10 random areas were selected in each slide: if Calcium compounds were detected in $0<x\leq4$ areas, the sample was classified as low pressure MSM; otherwise, whenever Calcium compounds were present in $5\leq x\leq10$ areas, the sample was classified as high pressure MSM. Statistical analysis showed 95 % sensitivity and 90 % specificity for the histological method. The diagnostic performance of the raters showed a high agreement between the two raters.

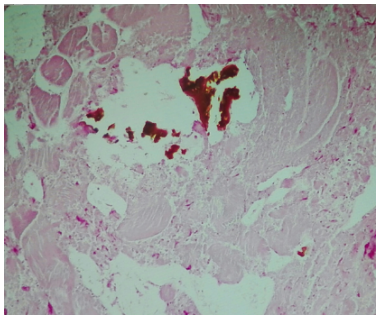

Figure 1. Chicken breast, *Von Kossa* stain. Calcium salts are highlighted in dark brown.

IV. CONCLUSION

This study proves that histology can be successfully used as a simple and economic tool to identify MSM from non-MSM products. Further studies (e.g., histomorphometry) could be set up in the future to identify low pressure MSM and high pressure MSM.

REFERENCES

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