Modification of muscle structure in poultry meat caused by different meat recovery systems

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Abstract—**Regulation (EC) No 853/2004, Annex I defines mechanically separated meat (MSM) in a complex way. MSM is produced from meat residues that adhere to bones after deboning. These residues are mechanically extracted which results in a disintegration of muscle structure, although this is not specified in detail. Currently, specific histomorphological analyses are missing in this field. The present study is to close this gap exemplarily for poultry meat.**

Meat samples were derived from a comparative study of different MSM systems. The samples were cut with a cryotom and stained with Toluidine Blue. Histomorphological assessment revealed a reasonable differentiation of four grades of disintegration of muscle fibres. They can be distinguished by the morphology of nuclei, occurrence of metachromatic stain, and the appearance of cross striation. Additionally, the integrity of connective tissue was considered. However, appropriate training is necessary for correct evaluation.

In summary, this assessment method can distinguish the effects of different mechanical deboning systems.

Keywords— **mechanically separated meat, poultry, histo-chemistry**

I. INTRODUCTION

Regulation (EC) No 853/2004, Annex I, 1.14 defines mechanically separated meat (MSM) in a complex way:

1. MSM is "obtained by removing meat from flesh-bearing bones after boning or from poultry carcases".
2. These meat residues are extracted "using mechanical means".
3. The extraction results in "loss or modification of the muscle fibre structure", although this is not specified in detail.

In contrast, this regulation defines *meat preparations* as "fresh meat, including meat that has been reduced to fragments, … which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the charateristics of fresh meat" (Annex I, 1.15). According to the perspective of this regulation, muscle fibre structure is of superior importance to differentiate between MSM and meat preparations. Therefore, the present study was conducted with the aim to characterize the histological changes that occur due to mechanical extraction, and to quantify these changes based on well identifiable charateristics.

II. MATERIAL AND METHODS

The material used for meat extraction were carcasses and legs of chicken, turkey thighs, as well as backs and carcasses of chicken (with remaining meat after treatment with TWD8, see below). For meat extraction, the following methods were used (for details see [1]):

- Two-phase system TWD8/Mado – a gentle method: combination of TWD8 (8-mm drum, 3-bar pressure, capacity 3000 kg/h; High Tec, Chapecó, SC, Brazil, after a technical development of Dr. J. Degenhardt from Perdigão, Videira, SC, Brazil), and a Mado automatic grinder, model Ultra 4 (4-mm drum, 5-bar pressure, capacity 3000 kg/h; Maschinenfabrik Dornhan GmbH, Dornhan, Germany)
- POSS separator model PDX5, modified – a gentle method: 3-mm drum, >100 bar pressure, capacity 5000 kg/h (POSS Ltd., Toronto, Canada)
- POSS separator model PDX5, original – hard separator: 0.6-mm lamellae, >250 bar pressure, capacity 5000 kg/h (POSS Ltd., Toronto, Canada)

For histological examination, frozen sections (10 µm, cryostat at -25°C to -40°C) were stained with the following method:

- 0.03% Toluidine Blue O (C.I. 52040) in aqueous solution for 10 min [2], rinsed in water and then covered with Karion F (Karuthin E, Art. No. 1123, Fa. Ruth, Bochum-Wattenscheid, Germany)
- Alcian Blue 8GX in critical electrolyte concentration of 0.6 mol/l $MgCl_2$ for preparation of acid glycosaminoglycans [3]
- Periodic Acid Schiff reaction (PAS) [4]

In a first, purely descriptive step, the sections were evaluated with the aim to differentiate tissue types that occur in the sample material, and to identify indicators of pressure-related modifications. In a second step, the sections were evaluated semi-quantitatively for the presence of certain microscopic standards according to the disintegration grade of muscle fibres (Table 1) as described by [1]. The histological appearance of the standards is exemplified in Figure 1. Complete sections with a standardized area of 1.8 x 2.2 cm^2 were evaluated at 100-fold magnification in about 100–150 fields of view. The classification of a field of view was decided by the worst standard that could be detected. The combined result for each section was expressed as the proportion of standards for all fields of view. The final result was calculated as the average of three sections per sample. For an initial assessment of operator effects, the evaluation was made by two persons: one trained histologist, and one with little experience after a short 2-h briefing.

Table 1: Definition of disintegration grades for microscopic evaluation of histological sections [1]

Standard	Characteristics	Occurrence
1A	Completely free of modifications	Uncritical effects of all deboning methods (incl. minced meat)
1B	Slight modifications of single fibres without affecting the structural grid of the muscle fibres	
2A	Slight traces of grinded fibres, inconspicuously located cell nuclei	
2B	Clear traces of grinded fibres, inconspicuously located cell nuclei	
3A	Grinded fibres with highly aggregated cell nuclei, attached to intact fibres, slight metachromasia (red instead of blue staining)	Severe technical effects of deboning (poor quality)
3B	Massively grinded fibres with highly aggregated cell nuclei in broad fringes, strong metachromasia (partly from squeezed-out cartilage), but remains of cell nuclei still recognizable as such	
4	Complete disintegration of the structure with very strong staining of the material, squeezed-out matrix of cartilage with severe metachromatic effects	Only in MSM

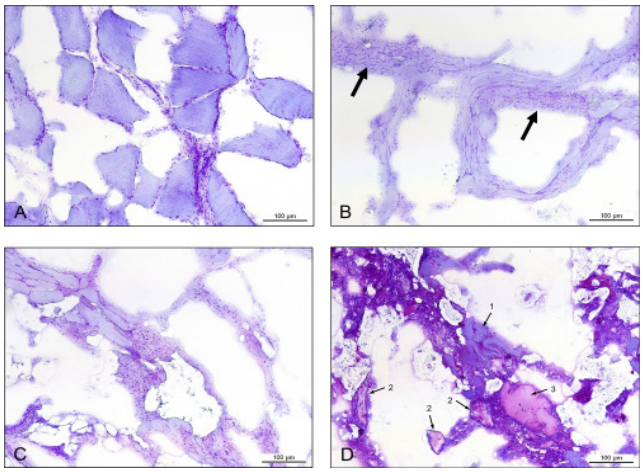

Figure 1: A: Standard 1 with fully preserved structure of muscle fibres and perimysium. B: Standard 3A with highly modified muscle fibres (dense accumulation of cell nuclei, pressure-induced changes of cell nuclei) that attach tightly to intact fibres (examples marked with arrows). C: Standard 3B – stronger modifications compared to Standard 3A, with slight metachromatic effects. D: Standard 4 – amorphous areas with strong metachromasia, muscle fibres with visible striation (1) but atypical staining, numerous particles of cartilage (largest marked 3), single bone fragments (2), partly remains of connective tissue; this standard can be clearly identified simply because of the lucid pressure-related stainability of the material. (All preparations with Toluidine Blue; original magnification 200x.)

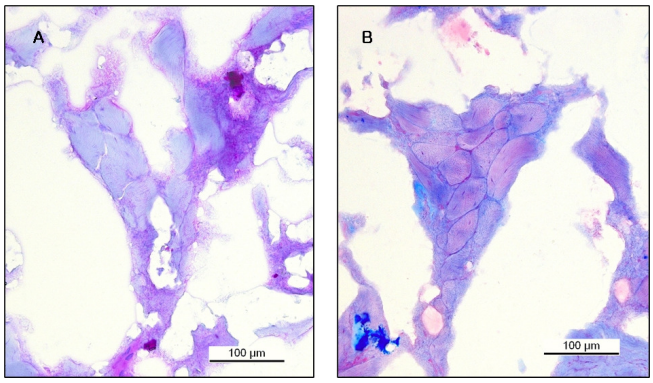

Figure 2: Imbibition of muscle tissue with glycosaminoglycans (GAG) of cartilage matrix. A: Metachromatic reaction (magenta) in Toluidine Blue staining. B: In the same area (adjacent section) specific detection of GAG (blue) with Alcian Blue (MSM from POSS original separator, chicken carcasses).

III. RESULTS AND DISCUSSION

A. Histomorphological evaluation of tissue types occurring collateral to muscle tissue

Besides muscle, different tissue types were found in the samples that were to be expected according to the type of raw material. Regular findings were skin, fat, tendon, dense connective tissue, bone, cartilage and large blood vessels. In connection with skin, scattered feather roots were found. In samples of turkey and chicken thighs, rather thick myelinated nerve fibres were regularly found. Noteworthy, these fibres were strongly stained by the PAS reaction that appears to be characteristic for myelin sheats in frozen sections [5]. In Toluidine Blue staining, nerve cells with Lipofuscin pigment were regularly identified in these fibres. Both characteristics are easy to identify and may be useful for the evaluation of MSM samples from other species. For chicken backs, parts of renal tissue were identified by characteristic renal corpuscles. Scattered cartilage particles were found in samples of all experimental groups. In samples of the gentle extraction procedures, they were present as discrete particles, some with artefacts that were rather due to the cutting technology than to the previous treatment. In contrast, extraction with very high pressure (POSS 0.6 mm, pressure >250 bar) resulted in characteristic changes:

- Fragmentation of cartilage particles to small fragments of ca. 10 µm diameter, distributed throughout the tissue. The mere occurrence of such fragments qualified for a tentative diagnosis of MSM.
- Squeezing out of the cartilage matrix and imbibition of surrounding tissue with “free” glycosaminoglycans (GAG). These “free” GAG, i.e. not bound to cartilage, could be identified by their strong metachromasia in Toluidine Blue staining and by their specific staining with Alcian Blue (Figure 2). The occurrence of these metachromatic areas was seen as indicative for MSM. However, imbibed areas with no further changes in muscle fibre structure were found as an exception also in individual samples from gentle extraction methods.

B. Semi-quantitative evaluation of muscle fibre structure

The semi-quantitative evaluation was carried out by two histologists with different expertise (Figure 3). Nevertheless, a generally good agreement was achieved in the differentiation between treatments, taking into account that the distinction between standards 1-2 (predominantly without pressure damage of muscle fibres) and 3-4 (considerable to severe modifications caused by pressure) is essential. For one sample (chicken legs, POSS low-pressure), two batches were available for analysis. Both batches were evaluated in the same critical way by both histologists. Similarly, the three problematic samples (groups 4, 5, 6 in Figure 3) were classified with a considerable proportion into Standard 3-4. In these groups, major muscle disintegration — and consequently this result — was to be expected due to the low quality of raw material in terms of a high proportion of bone, combined with high pressure treatment. Meat extracted from chicken carcasses with a hard separator (group 6) was classified worst by both histologists. Overall, however, there was a tendency to stricter assessment by histologist 2. In samples from turkey drumsticks, this led to severe, non-negligible deviations between the two evaluators, and they differed even for minced meat (group 1 in Figure 3). In these samples, a high proportion of bone tendons and of dense connective tissue may have made the evaluation too difficult without appropriate training.

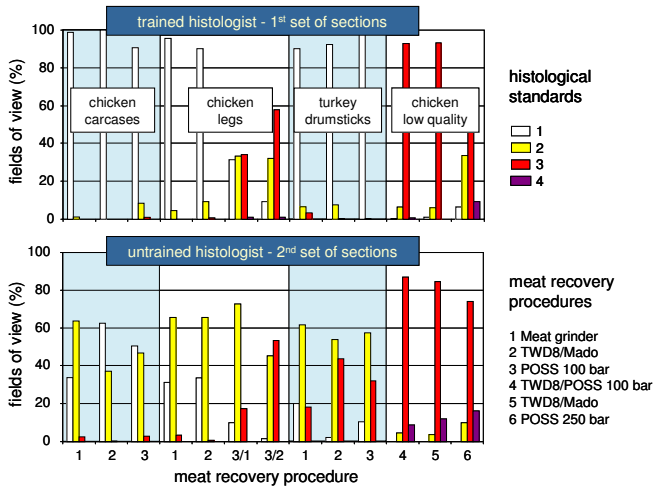

Figure 3: Histomorphological evaluation of sections according to histological standards by two histologists with different expertise (the section sets were from identical samples; for low quality chicken, the raw material was: 4 and 6 carcases, 5 backs)

IV. CONCLUSIONS

For the differentiation of the various stages of disintegration of muscle fibres, the histological staining of frozen sections with Toluidine Blue O is a suitable method. With only one staining step and inclusion in an aqueous medium, the method is technically fast and easy. As a drawback, sections can be preserved but for a few months.

The staining displays the cell nuclei of all tissues in strong extinction, and cytoplasmatic structures in light-blue background staining. As a special advantage of the method, the typical metachromatic red stain component becomes more prominent with increasing disintegration. Consequently, in MSM, areas with the strongest structural modifications exceptionally emerge in a lucid range of colours between light blue and bright red. Further staining methods may be applied for additional information. The evidence of highly acid GAG by Alcian Blue (with critical electrolyte concentration) confirms the detection of squeezed-out cartilage matrix, which is important for the diagnosis of MSM.

For poultry, the reliable analysis of myelinated nerve fibres with the PAS reaction may be of minor importance in this context, but it could be of benefit in studying MSM from other types of meat.

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