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Performance of conventional histochemical methods relative to a novel immunolabeling technique in assessing degree of degradation in comminuted chicken meat

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The potential of a novel immunohistochemical method detecting the muscle protein meromyosin and the basement membrane protein laminin to objectively and accurately determine the degree of degradation of muscle tissue in comminuted chicken meat was investigated in comparison to three different conventional methods, two slightly different toluidine blue (TB) staining procedures and a haematoxylin-eosin (HE) staining. All samples were evaluated for degree of muscle degradation, one method by image analysis system and three by visual assessment of degree of degradation. Analyses were carried out on reference samples consisting of mixtures of emulsified and minced meat in steps of 10%. The immunohistochemical approach (LAM) showed better selectivity and good image quality. The correlation (R) between the amount of minced meat in mixtures and degree of degradation were higher and consistent by this approach (0.94 compared with 0.62, 0.61, and 0.85 for thigh muscles by LAM, HE, TB-Max Rubner-Institute (MRI) and TB-Leatherhead Food Research (LFR), respectively, and 0.91 compared with 0.73, 0.73 and 0.98 for breast muscle by LAM, HE, TB-MRI and TB-LFR, respectively). When applied to industrial samples, the results showed that it is possible by all methods to separate at least two different groups based on degree of degradation. In conclusion, the good image contrast, specificity of the staining and objectivity and transparency of the measurement speaks in favor of the LAM method as optimal to quantify degree of degradation of muscle structure in comminuted and fragmented chicken meat and obviously a likely candidate for a standard method.

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1. Introduction

Worldwide consumption of meat and meat products is progressively growing and is expected to increase 30% by year 2050 (Alexandratos & Bruinsma, 2012). Subsequently, the tendency to produce and consume chicken and chicken products will most likely increase as well, and result in higher demand for deboned products in comparison to whole chicken. This will imply relatively higher concomitantly increase in the amount of carcasses and carcass parts with varying amounts of meat still attached to the bones and carcass parts. This meat can be recovered either by hand or by mechanical means and both these procedures will affect the structure of muscle fibres. Muscles are highly organized tissues, consisting of bundles of muscle fibres surrounded by connective tissue in which blood vessels and nervous tissues are embedded for nutrient supply and function. Degradation of muscle fibre structures involves separation of individual fibres and fibre bundles as well as degradation of individual fibres and their surrounding membrane (sarcolemma and basal lamina). In general all handling of meat implies a degradation of muscle fibre structure whether it is ageing, mincing, freezing, thawing, marinating and so forth primarily to enhance functionality or tenderness of the meat. If the meat from the carcass parts is recovered by hand the product is considered as meat and legally treated as such. If, however, the

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recovery is done mechanically the product should be regarded as mechanically separated meat (MSM) and treated as such in accordance to present EU legislation (Regulation No. 853/2004). Legislation thus assumes the degree of degradation of muscle fibres in the mechanically separated meat is more pronounced hence meat is of lower quality. Currently no method has been provided by which the degree of degradation of muscle tissue can be measured accurately and objectively and as such no threshold levels for different degradation classes exist nor can the lower quality assessment of MSM due to degree of degradation be documented.

By the deboning procedure, muscle of breast, leg and wings are cut from the carcass. To recover the meat from the remaining parts of the carcass for practical and economic reasons mechanical separation procedures are necessary. Within the industry there is an increasing interest in the degree of degradation of the muscle fibre structure as it is part of the quality and functionality of the products. This has led to quality differentiation by methods mainly focusing on chemical composition of the product (protein, fat, connective tissue and Ca^{++}) but also histochemical approaches have been developed that further include degradation of muscle structure (Branscheid, Bauer, & Troeger, 2011; Groves, 2011; Sifre, André, & Coton, 2009), this has led to variations in the market price of the different qualities.

A rapid development of machines for separation has indicated that some MSM products have a muscle fibre structure that cannot be distinguished from that of regular minced meat. Still the prize difference between MSM of least degradation and minced meat is rather dramatic, mainly due to legislation. Implementation as well as interpretation of the different regulations covering MSM in the different EU countries also vary considerably (EFSA, 2013) and have led to uneven competition within EU. Therefore, there is a collective compulsion from meat industry, European Food Safety Authority (EFSA) and legislative bodies within the EU to establish an objective and accurate method to quantify the degree of degradation of muscle structure.

In the present work, three histological approaches and one immunohistochemical approach were tested for determination and accuracy of degree of degradation of muscle structure: 1) Toluidine blue (TB-Max Rubner-Institute (MRI)) (Branscheid et al. 2011), 2) Haematoxylin-eosin (HE) 3) Toluidine blue (TB-Leatherhead Food Research (LFR)) with a different staining methodology compared with TB-MRI and, 4) immunostaining for meromyosin (MYO) and laminin (LAM). These 4 methods were used on two types of materials: 1) reference material consisting of 11 mixtures of varying levels of minced and emulsified material from breast or thigh muscle to test the linearity of the methods and 2) industrial samples to visualize the range of degradation levels in MSM products. The methods were compared based on the capability of predicting degree of degradation in the reference samples and on the ranking of the industrial samples.

2. Materials and methods

2.1. Materials for staining

Toluidine blue O and eosin were purchased from Merck (Darmstadt, Germany), haematoxylin from Shandon, (Pittsburgh, PA, USA); meromyosin MF-20 antibody, deposited by Fischman, D.A., and laminin 31-2 antibody, deposited by Fambrough, D.M., from Developmental Studies Hybridoma Bank (University of Iowa, Department of Biology, Iowa City, IA).

2.2. Sample preparation

The meat samples were collected from commercial broiler lines 35 days old (Ross 308) from two commercial slaughter-houses in Denmark and used for the reference samples, industrial samples and the hand-deboned samples. Two sets of reference samples of 11 mixtures with varying amounts of minced and emulsified meat from breast (without the inner fillet) and thigh muscle (deboned thighs), respectively, were produced. The whole muscles were either coarsely chopped through a plate with 5-mm holes using a commercial mincer (Bankeryd, Sweden) or emulsified for 8 min using a commercial grinder (Mado, Germany). Both sets consisted of mixtures from 100% of minced and 0% emulsified meat to 0% of minced and 100% emulsified meat in 10% units. In addition, 12 industrial MSM samples including low, medium and high pressure MSM types of meat and also different raw materials as input to the machines to cover the entire range of degradation (Table 1) were used in the testing of the methods. Furthermore, two hand-deboned samples, minced using a 5-mm-hole plate followed by a 2-mm-hole plate from breast and back parts were included in the study (Table 1). At the day of sample collection the samples were frozen and stored at −20 °C before shipping to the different laboratories. In all 36 samples were delivered frozen in hermetically sealed bags for histological analysis in three laboratories, in a non-identifiable abbreviation. The number of samples and the number of areas to analyze for the individual samples were based on results from preliminary investigation on reference samples using repeatability models of Becker (1992).

2.3. The histochemical methods carried out by MRI

For histochemical investigation, the samples were thawed in a cooling chamber at 4 °C for 12 h, agitated with a spatula to ensure homogeneity, and then mixed with mounting medium (6 wt%) (Tissue tek, Leica, Germany) to assure adhesion of the material. Twenty g of the subsequent material was divided into 8 aluminium foil cups, frozen by immersion in liquid nitrogen-cooled isopentane, and stored at −20 °C until further examination. The samples were cut in a cryostat (Leica CM3050, Germany) at −20 °C into 12 μm thick sections. Frozen sections were collected on glass microscope slides (two sections per slide), and kept at room temperature. Toluidine blue (TB-MRI) staining was performed according to Branscheid et al. (2011) by immersion in a 0.03% TB solution. For HE staining, first a solution of 0.6% haematoxylin and then a solution of 0.1% eosin was used for submerging the slides. The coverslips were fixed with a water-insoluble histofluid (Eukitt). Microscopic work was conducted on a Leitz Wetzlar transmitted light microscope equipped with a digital color CCD camera (0.5x: 1/2) (Leica EC3, Germany). The objective was PL APO 16x, NA 0.40, and image resolution 2048x1536. There were eight replicates and at least five slides per replicate, and three images per slide.

The MRI approach used a modified method according to Branscheid et al. (2011) to assess the acquired images: the rating of MSM was given in a 4-point scale (1 as excellent structure, 4 as loss of structure). In the present case, the evaluation was presented also as a percentage of structured fibres in a sample, thereby generating continuous data in order to compare with the other methods. This was applied for both TB-MRI and HE staining.

2.4. The histochemical method carried out by LFR

At LFR the frozen samples were thawed out slowly and gently agitated within the bag to incorporate any separate liquid. Small amounts of samples (1 g) were placed on a metal stub using a mountant (Tissue tek, Leica, Germany), and frozen by immersion in liquid nitrogen. The samples were mounted in a Bright 5030 cryostat at −23 °C. Sections with 10 μm thickness were collected on glass slides, and stored at room temperature until further

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