

The microstructure of whole-muscle meat products

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Abstract

This paper describes the microstructure of whole-muscle meat products. It describes the microstructure of hams of the highest quality, choice hams, standard hams, and other whole-muscle products including dry-cured hams and poultry hams. The histological characterisation of pork ham of the highest quality and choice ham indicates that skeletal muscle forms demarcated areas connected by a protein matrix formed of released muscle proteins. Sites of brightening following injection may be evident in the muscle fibres, particularly on longitudinal cuts. In standard hams, skeletal muscle forms smaller, clearly demarcated areas with a larger proportion of protein matrix. Hydrocolloids (native and modified starch, carrageenans, xanthans and plant proteins) are a commonly detectable part of the protein matrix in standard hams. Other whole-muscle products that do not fall under the definition of ham have clearly demarcated large areas of skeletal muscle, with food additives of plant origin detectable in the protein matrix. Closer connections between the muscle fibres are evident histologically in the group dry-cured hams. The skeletal muscle in these products is, however, subject to the smallest degree of histological change.

Dry-cured ham, ham, histology, microscopy

Introduction

This paper describes the microstructure of whole-muscle meat products. This is the group of meat products that is the most homogenous in terms of microstructure, although certain differences, which are usually the result of differing methods of production, can be found. Salting, tumbling and the use of additives have an effect on microstructure. Whole-muscle meat products can be defined from the microscopic viewpoint as products comprised predominantly of skeletal muscle, with a smaller proportion of fat tissue (Plate III, Fig. 1) and associated connective tissue (Plate III, Fig. 2). Heat-treated whole-muscle meat products are encountered most frequently in the Czech Republic. According to the legislation, this means ham which falls into the quality classes – ham of the highest quality, choice ham and standard ham. This classification is performed on the basis of the content of pure muscle protein. The use of fibre, starch, plant and other animal proteins is not permitted in the first two of these quality classes. The use of additives in standard hams is reflected in the microstructure of these products which is described below.

The microstructure of whole-muscle meat products - the histological characterisation of ham of the highest quality and choice ham

Pork ham of the highest quality (Plate III, Figs 1 and 3) and choice ham (Plate III, Figs 2 and 6) may, according to the legislation, be made only from whole-muscle leg of pork (Decree No. 326/2001). Under an optical microscope, clearly demarcated areas of skeletal muscle can be seen clearly connected by a protein matrix formed of released muscle proteins (Plate III, Figs 1 and 3). Sites of brightening or small cavities created during injection may be evident in the muscle fibres, particularly on longitudinal cuts (Plate III, Fig. 5). These are not visible to the naked eye if the correct injection technology is used, suitable brine selected and high-quality ingredients used. Cavities larger than 0.2 mm are visible

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macroscopically and may be perceived negatively by the consumer. Image analysis techniques can be used for the analysis of macroscopically visible cavities similarly as for microscopic slides (Valous et al. 2009 and 2010). Microscopic changes may also differ according to the type of muscle injected (Krzywdzińska-Bartkowiak et al. 2016).

The histological characterisation of standard ham

In standard pork ham, for which the legislation permits the use of grained leg of pork, and in poultry ham, the muscle tissue is arranged in smaller areas, for which reason smaller areas of clearly demarcated skeletal muscle and a larger proportion of the protein matrix that connects these areas are evident under the microscope (Plate III, Fig. 4 and Plate IV, Fig. 7). Hydrocolloids are also a common finding in this group of products. These are used primarily to improve water binding, though also to regulate texture parameters. A number of studies have described their impact on colour, toughness, yield, sensory properties and the reduction of water loss. They are usually added to the brine, for which reason they generally make up part of the intermuscular spaces (filler) (Plate IV, Fig. 8) between the individual pieces of muscle tissue. Starches (Plate III, Fig. 4 and Plate IV, Fig. 8) (Resconi et al. 2016) or altered forms such as modified starches are also widely used; falsification may also take place (Plate III, Fig. 6). Carrageenans are also used and have been shown to have a positive effect on ham hardness following their application (Verbeken et al. 2005), as well as on water binding and the stability of the meat emulsion in turkey meat products (Ayadi et al. 2009). Less widely used are xanthans and plant proteins such as, for example, soya protein (Plate IV, Fig. 9) which may, in combination with image analysis, also be assessed quantitatively (Randulová et al. 2011). Currently, the use of various combinations is encountered most frequently in view of their frequent synergetic effect. The combination of starches and carrageenans is probably the most common. However, as has been shown by Prabhu and Sebranek (1997), interaction between the molecules of these additives has not been proven by microscopic methods.

The brine may also contain NaCl, polyphosphates, nitrite salt and permitted flavour enhancers, usually monosodium glutamate. Their microscopic identification using an optical microscope is not, however, possible due to their solubility in water (Pospiech et al. 2014). Their prediction is possible with the use of other imaging methods. An example of this is provided by the study by Fulladosa et al. (2010) who used an imaging technique based on computer tomography (CT). The precision of this method was 0.3% for salt and 1.5% for water content. With the development of new technology, however, such methods are now capable of achieving higher resolutions down to $1\ \mu\text{m}^3$ in methods known as μCT (Landis and Keane 2010). The principal advantage of these methods is that they are non-destructive. They can, therefore, be used similarly to metal detectors in the inspection of individual product items, and enable the analysis of a large number of parameters on the basis of varying absorption of x-ray radiation. The results produced by μCT in particular can also be used to estimate textural properties. A positive correlation has been demonstrated between μCT analysis and hardness, though it has not been possible to detect fat in the emulsion phase (Santos-Garcés et al. 2013). For whole-muscle products, in which there is a low proportion of filler and, thereby, a low proportion of emulsion forms of fat and proteins, this should not represent a problem during analysis.

The histological characterisation of other whole-muscle products

Other whole-muscle products that do not fall within the definition of ham according to the legislation (Decree No. 326/2001) include, for example, Debrecen ham, garlic ham, bone-in ham and smoked meat. These products do not, therefore, have defined limits for the use of additives under the condition that they are permitted for use in meat products.

For this reason, such additives are commonly encountered in these products and can be demonstrated under the microscope. Similar additives are used as in standard ham. The structure of the muscle tissue remains intact and forms large and sharply demarcated areas. Plant additives become part of the protein matrix that connects the muscle areas similarly as in standard ham (Plate IV, Figs 11 and 12) (Tremlová et al. 2013).

The histological characterisation of dry-cured hams

Dry-cured hams are another popular group of whole-muscle product. This type of product is particularly popular in Spain and Italy, though it can also be found in the Czech Republic. The microstructure of these products is different to that of cooked whole-muscle products. These products most closely resemble the original structure of muscle tissue and are characterised by close connection between the individual muscle fibres (collagen connective tissue unsheathing the individual muscle fibres) by the endomysium. The myofibrils (fibres of contractible proteins) are firmly attached to one another and are also connected with the sarcolemma (cell membrane). Other changes described are associated principally with the production technology and involve changes arising primarily during the salting phase, reaching a peak following product ripening, and they differ according to the type of muscles used and are more pronounced in *m. semimembranosus* than in *m. biceps femoris*. Another change seen is a shift in the Z-line, which is no longer on one level, while pronounced degradation of the sarcolemma is also seen. These changes have been studied by transmission electron microscopy (TEM). At the end of resting, the formation of dissolved substances in the muscles can be observed as the result of proteolysis (Larrea et al. 2007a). A less pronounced Z-line and transversal damage to muscle fibres regardless of the muscle used are also described with the use of optical microscopy by Monin et al. (1997). These changes are multiplied following salting and resting. The M-band is evident at the end of resting only in *m. biceps femoris*. It is not evident in *m. semimembranosus* (Monin et al. 1997). Microstructural changes are also described in fat tissue. In intramuscular fat, salting results in the release of part of the fat from the fat tissue and its subsequent attachment to form small areas of free fat in intercellular spaces. Infiltration of the muscle tissue by free fat and products of lipolysis occurs during subsequent drying. This process is also important to change in sensory properties. In subcutaneous fat, penetration of the salt solution among the originally close connections of the fat cells occurs during the salting process through the collagen connective tissue, which leads to the formation of a large intercellular space and infiltration of individual cells. The loss of water from the cells and the release of fat into the intercellular space then occur. In certain cells, plasmolysis can also be seen, which also leads to the release of fat into the intercellular space (Larrea et al. 2007b).

Description of used histological staining

Haematoxylin and Eosin (HE)

This is a basic staining that is widely used in classical histology and pathological medicine. The advantage of this staining from the viewpoint of food microscopy is the fact that it stains the majority of animal cells and tissues with its two components, natural Haematoxylin and artificial Eosin. Its use on plant cells and tissues is limited, primarily due to its inability to stain plant polysaccharides in cell walls and the limited stainability of plant assimilates, with the exception of protein inclusions (aleurone grains). Staining results in blue cell nuclei, red sarcoplasm in skeletal muscle and light pink collagen connective tissue in dry-cured and other non-heat-treated hams. In cooked hams, the collagen connective tissue is stained blue. Plant cells and inclusions are less pronounced.

Calleja

This is one targeted staining serving to demonstrate collagen connective tissue. Targeted stains, including Calleja staining, highlight a given structure over other tissues in view of differing stainability or different contrast. The advantage of this staining is its speed and the homogeneity of the results it produces. It is a staining that can easily be combined with other stainings. The result of staining is collagen connective tissue coloured blue and muscle tissue coloured green. Cell nuclei are coloured red. Plant cells and inclusions are less distinctive, with the exception of protein inclusions which are stained green to blue depending on the type of protein.

PAS Calleja (Periodic Acid Schiff's Calleja)

This is a combined targeted stain for demonstrating plant cells, tissues and saccharide inclusions. This staining highlights two basic structures – polysaccharides and collagen connective tissue. Collagen connective tissue is stained by the above-mentioning Calleja staining and polysaccharides by the second PAS component. The principle behind the staining of polysaccharides is the chemical reaction of oxidation (1,2-hydroxyl groups of hexoses, 1-hydroxy-2-amino- 1-hydroxy-2-alkylamino, 1-hydroxy-2-keto groups) with an acid. Oxidation produces aldehydes whose presence is shown by the Schiff agent. The formation of pink, red to purple-red structures is considered a positive PAS reaction. The advantage of this staining is the opportunity it provides of investigating constituents of both animal and plant origin. Staining results in collagen connective tissue coloured blue and muscle tissue coloured green. Cell nuclei are coloured red. It is worth noting that animal tissues containing a larger amount of polysaccharides, such as the mucous glands or the goblet cells in the intestines, also show a positive PAS reaction. Plant cells and polysaccharide inclusions are coloured pink. Protein inclusions are coloured pink-green. The intensity of staining depends on the content of aldehyde bonds and the presence of polysaccharides in the inclusion.

Conclusions

The histological characterisation of pork ham of the highest quality and choice ham indicates that skeletal muscle forms demarcated areas connected by a protein matrix formed of released muscle proteins. Sites of brightening following injection may be evident in the muscle fibres, particularly on longitudinal cuts. In standard hams, skeletal muscle forms smaller, clearly demarcated areas with a larger proportion of protein matrix. Hydrocolloids (native and modified starch, carrageenans, xanthans and plant proteins) are a commonly detectable part of the protein matrix in standard hams. Other whole-muscle products, including Debrecen ham, garlic ham, bone-in ham and smoked meat, are characterised by clear histologic differences from the previous groups. The skeletal muscle in these products is clearly demarcated and forms large areas, and food additives of plant origin are detectable in the protein matrix. Various food additives are used in view of the fact that there are no legislative restrictions. Closer connections between the muscle fibres are evident histologically in the group dry-cured hams. The skeletal muscle in these products is, however, subject to the smallest degree of histological change. The used histological staining indicates that Haematoxylin Eosin staining and Calleja staining are suitable for the characterisation of microstructure. These stainings are not, however, suitable for determining additives of plant origin, though these are detectable in the protein matrix with PAS-Calleja staining.

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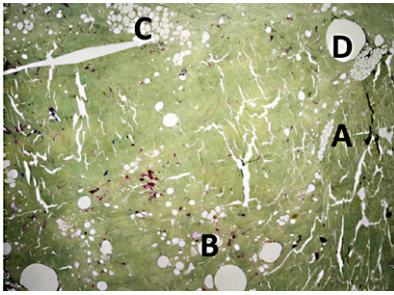


Fig. 1. Ham of the highest quality - Calleja, 40x
A skeletal muscle, B protein matrix, C fat tissue,
D air pocket

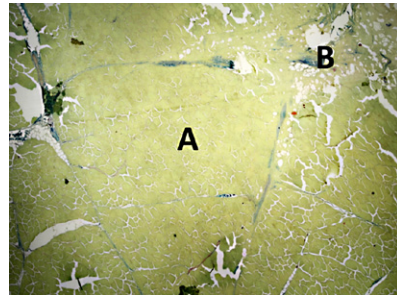


Fig. 2. Choice ham - Calleja 100x
A skeletal muscle, B collagen connective
tissue - blue

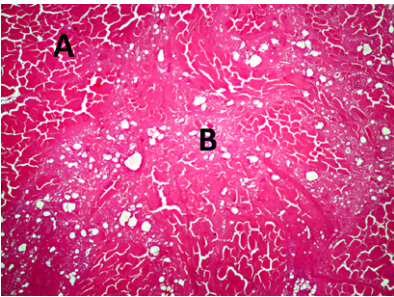


Fig. 3. Ham of the highest quality - HE, 40x
A skeletal muscle, B protein matrix

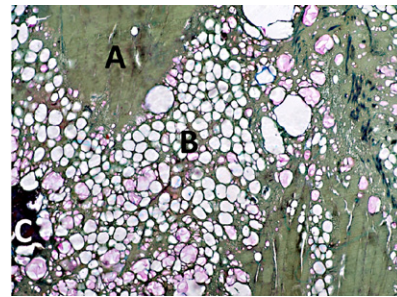


Fig. 4. Standard ham - PAS Calleja 100x
A skeletal muscle, B protein matrix with
starch - pink, C collagen connective tissue



Fig. 5. Choice ham - Calleja
A muscle fibres, arrow cavities following
injection

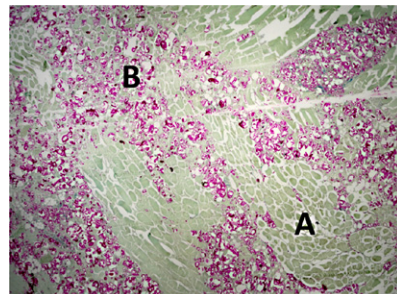


Fig. 6. Standard ham - PAS-Calleja
A skeletal muscle, B protein matrix with
starch - pink

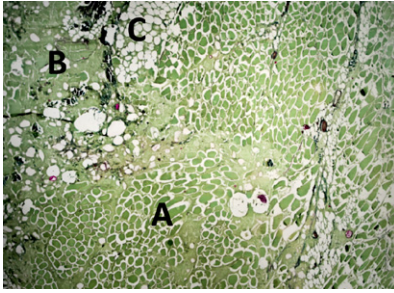


Fig. 7. Standard ham - Calleja, 40x
A skeletal muscle, B protein matrix, C fat tissue

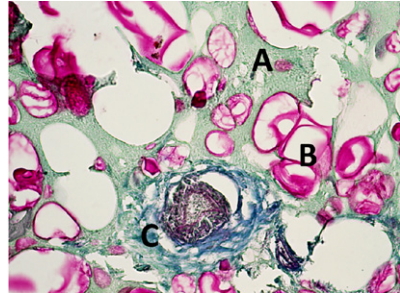


Fig. 8. Standard ham - PAS Calleja, 600x
A protein matrix, B starch, C collagen connective tissue

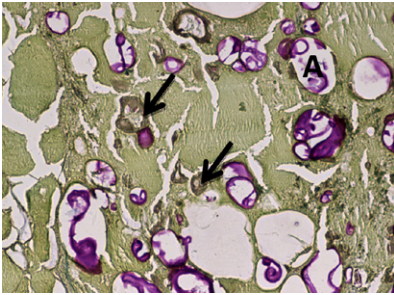


Fig. 9. Standard ham - PAS Calleja, 100x
A starch, B soya protein

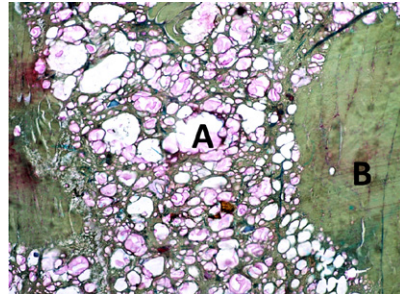


Fig. 10. Standard ham - PAS Calleja, 400x
A starch, B skeletal muscle

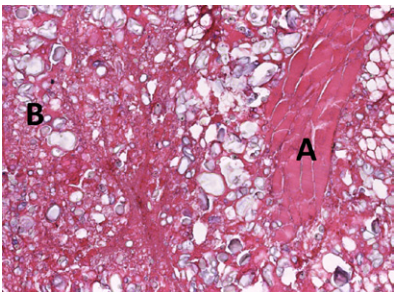


Fig. 11. Toast ham - HE, 100x
A muscle tissue, B starch grains in the protein matrix - the starch is darker

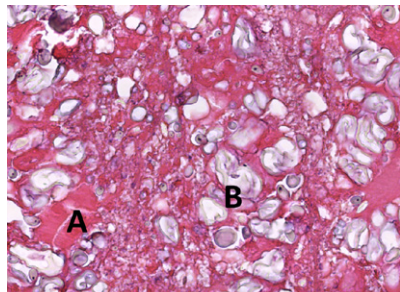


Fig. 12. Toast ham - HE, 100x
A muscle fibre, B starch grains in the protein matrix - the starch is darker