Are intermediate filament proteins involved in determining meat quality?

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Introduction

Biochemical studies have shown that desmin and vinculin are degraded with conditioning in bovine [1-3], porcine and chicken meat [4]. These findings have led to speculation that the degradation of intracellular cytoskeletal (intermediate filament) structures is involved in meat tenderisation. Degradation of the intermediate filament (IF) network may tenderise meat by easing the separation of myofibrils, thereby weakening the lateral structure of meat [1] and degradation of costameres may weaken the sarcolemma, thereby increasing calcium influx and calpain activity [3].

The current model of changes in water-holding capacity in muscle post-mortem involves lateral shrinkage of myofibrils as the primary mechanism driving water loss. Lateral shrinkage of myofibrils, muscle fibres and entire muscle fascicles, generates drip channels between fascicles. This mechanism requires that lateral connections formed by IFs through the costameres to extracellular structures are sufficiently strong to withstand displace fluid released by shrinkage of individual myofibrils to the extracellular spaces.

IF proteins may therefore be implicated in variations in the two most important quality parameters in meat; tenderness and water-holding. Degradation of IFs by post-mortem proteolysis may explain time-dependent changes in both of these parameters. There is evidence [5] that degradation varies between muscles. This may be due to variations in proteolysis between muscle fibre types. The aim of this study was to investigate IF stability during post-mortem conditioning of porcine meat by immunofluorescent labelling techniques and whether these showed any relation to changes in texture and water-holding measured by classical techniques. Changes in IF immunoposivity are related to muscle fibre type and compared between two muscles. This study is related in greater detail in a recently submitted paper [6].

Materials and Methods

Six pork loins were obtained from commercial weight (110kg) Danish pigs within 45 minutes of slaughter. Longissimus muscles were taken from all six loins for immunohistochemistry, pH, Warner Bratzler and water-holding capacity measurements. The redder iliocostalis muscle was obtained from three of the loins for comparison with the longissimus.

All samples were stored for 24 h at 15°C and then at 4°C. Tenderness, pH and water-holding capacity measurements were taken on days 1, 2, 4 and 7 except for Warner-Bratzler shear force, which could not be determined on day zero, or on the small iliocostalis muscle. Water holding capacity was measured by drip loss from suspended slices [7] and also by the filter-paper press method.

Cryo-sections of muscle were taken on days 0, 1, 2, 4 and 7 and incubated with monoclonal antibodies against desmin, vinculin, myosin type I, myosin type II and myosin type I+IIa. These were then fluorescently labelled with FITC conjugated rabbit anti-mouse as the secondary antibody, photographed on a Leica DMIRB inverted fluorescence microscope. Controls were performed either by using non-immune mouse immunoglobulin at the primary antibody stage or omitting the primary antibody completely.

Results

Meat quality parameters

During the entire storage period, all longissimus muscles continuously lost liquid through drip, with the highest losses occurring during the first day of storage (Figure 1). The time course is in accordance with other reported research [8]. In contrast to the gradual increase in drip losses, changes in the water-holding observed with the filter paper press method mainly took place during the first day of storage. The filter press results appear to be more directly related to myofibrillar water holding capacity than whole meat drip loss, where the time course of extracellular transfer of water and formation of drip channels are additionally involved in determining water loss. Less water was pressed out from the iliocostalis than the longissimus muscles at all storage times and the amount of expelled water in this muscle tended to increase through storage. The decrease in relative sample weight closely parallels the decrease in pH; the correlation between these parameters being 0.85.

Toughness as measured by WB shear force decreased during storage. The principal decrease in average WB shear force values occurred between day 1 and 2, and from day 4 little change was observed (Figure 1). However, substantial variations in WB shear force values were observed between muscles and between sub-samples from a muscle at a given storage time.

Immunohistochemistry

In contrast to the intensity of labelling of myosin isoforms, which remains high throughout the storage period studied, the intensity of immunolabelling against desmin and vinculin decreases with storage time in both longissimus and iliocostalis muscles. The loss of vinculin labelling at the periphery of fibres is fairly uniform throughout each muscle, but the rate of loss appears slower in iliocostalis than in longissimus muscle. Desmin labelling also declines more slowly in iliocostalis than in longissimus. In longissimus muscle it is clear that the loss of labelling is not uniform across the muscle; see figure 2. Desmin labelling appears to be preferentially lost from some fibres. Comparison with serials sections labelled against various myosin isoforms reveals that retention of desmin labelling is highest in type I and IIa fibres.

Discussion

This study is consistent with previous work indicating that IF and costamere proteins have a role in determining meat tenderness [3] and extends this by examining potential differences in IF degradation between muscle fibre type. The pattern of protein degradation is also consistent with the hypothesis that IF/costamere proteins may also be implicated in drip loss. The two proteins studied (desmin and vinculin) were taken as representative proteins in the exosarcomeric lattice and costameres, respectively. The slower loss of IF labelling in iliocostalis muscle than in longissimus correlates with previous work measuring the strength of single muscle fibres [9] which showed that the average strength of fibres from iliocostalis is considerably higher than from longissimus both raw and cooked material. Further studies are now underway required to test directly the mechanical integrity of IF and costamere structures on known fibre types, in order to show that degradation of IF/costamere proteins is a determining factor in meat tenderisation and drip development, and not merely a correlation to them.

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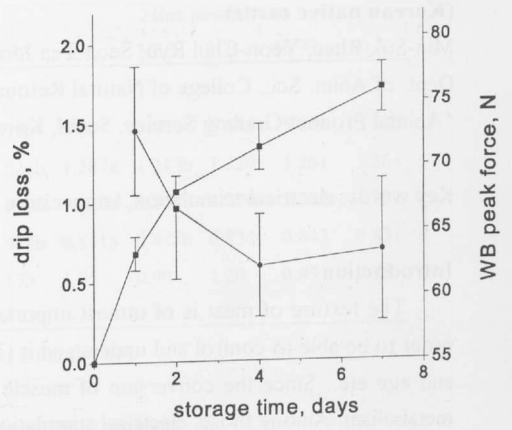

Fig. 1. Mean Warner-Bratzler shear force values (circles) and drip loss (squares) versus post-mortem storage time in porcine longissimus muscles. Error bars indicate ± one SE. For Warner Bratzler each point is the mean from 6 muscles. For drip, n=5; a sixth muscle had significantly higher drip losses than the rest and is not shown.

Acknowledgements

We acknowledge technical assistance from Claudio Moralis and financial support from the Danish Agricultural and Food Research Council and the Norma and Frode S. Jacobsen Fund.

Fig. 2. Composite micrograph showing (top row)type I myosin - labelled fluorescence images on 0,1,2,4, and 7 day samples (from left to right) together with anti-desmin - labelled images at the same times (middle row) and phase contrast images of the relevant sectional areas (bottom row). At longer storage time desmin labelling is preferentially retained in a minority of fibres which co-localise with I and IIa muscle fibre typing.