## The structural basis of drip loss from pork: role of cytoskeletal proteins

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### Background and aims

The capacity of fresh meat to hold its water is of major economical importance. 2-5% of the lean meat content of normal pig carcasses are lost during slaughter and storage at the abattoirs. Subsequent cutting, handling and storage during retail cause additional water losses. There is a large variation in the water-holding capacity (WHC) between pig carcasses. Some causes for this variability are well known. The WHC is affected by genetic defects such as PSE and the so-called Hampshire effect and by stress during transport and slaughter. However, these known factors are not sufficient to explain the observed differences in water losses. It appears that other yet unidentified factors are involved in determining the WHC of meat.

The current belief is that water is lost from meat by a two-step mechanism. First biochemical changes during the development of rigor mortis cause a rapid lateral shrinkage of myofibrils leading to shrinkage of whole muscle fibres and entire muscle fibre bundles and to the development of water-filled channels between the fibre bundles. Then water slowly drains via these channels to the surface of the meat leading to evaporation and drip formation.

The first step in this mechanism implies that intact and competent lateral links exist across the entire muscle. Intermediate filaments and other cytoskeletal structures form such coss-links and we believe that the integrity of these structures play a role in determining the extent of water losses. It has been demonstrated that cytoskeletal proteins are degraded by proteolysis during conditioning of this muscle (1,2). Degradation of the intracellular intermediate filaments may improve WHC by softening intracellular lateral structures, thereby reallocating water from the extracellular channels to the intracellular compartment, while proteolysis of costameres and extracellular links to connective tissues may increase water losses by widening the gaps between fibres.

The aim of our experiment was to ascertain that a modification of the rate of proteolysis of various cytoskeletal proteins (desmin, vinculin and the costameric protein talin) does affect the WHC of porcine meat as predicted by this hypothesis.

### Materials & Methods

The longissimus muscle from the loins of six normal slaughter pigs were obtained from a local abattoir and chill stored for up to seven days. pH and drip loss were measured on each day. The normal course of post-mortem proteolysis was modified by incorporating various divalent metal ions. $Zn^{2+}$ or $Ca^{2+}$ were used to depress or accelerate proteolysis, respectively, while $Mg^{2+}$ was used as an inactive 'blank'. The ions were applied either by incubating small meat cubes in buffers [blank: 8% PVP, 80 mM KCl, 3 mM $MgCl_2$, 3 mM ATP, 4 mM EGTA, 20 mM MES, pH 5.5; with $Zn^{2+}$: 8% PVP, 60 mM KCl, 8% PVP, 3 mM $MgCl_2$, 3 mM ATP, 4 mM EGTA, 20 mM MES, 10 mM $ZnCl_2$, pH 5.5] or by mixing the salts at 1 mM concentrations with ground meat. The degradation of desmin, vinculin and talin was followed by SDS-PAGE and immunoblotting (Western blots) and quantified by scanning of stained bands. WHC was measured by centrifuging raw, incubated or ground samples at 1000*g*. This method makes it possible to measure WHC in small samples without applying as large external pressures as in the filter-paper-press.

### Results

The development of water loss by centrifugation was similar to the development of drip loss in samples incubated in buffers, while it reached a maximum after one day of storage and then rapidly declined in the ground samples (Figure 1). The increase in WHC observed after one day of storage of ground samples is normally attributed to swelling and gelation of liberated fibres and fibrils.

According to the hypothesis, a faster degradation of intermediate filaments should increase the WHC, while slower degradation should increase water losses. Addition of $Zn^{2+}$ did significantly increase water losses in samples stored in buffers and ground samples, while no effect of $Ca^{2+}$ was observed (Figure 1).

The Western blots (figure 2 and 3) showed that all three cytoskeletal proteins disappeared during storage. The degradation rates of desmin and vinculin were similar with a significant loss during the first day post-mortem and a slower loss thereafter (Figure 2). Talin disappeared much more rapidly with only traces left in pre-rigor samples taken two hours post-mortem. The Western blots also showed a significantly slower degradation in $Zn^{2+}$-treated samples and a marginally faster degradation in $Ca^{2+}$-treated samples.

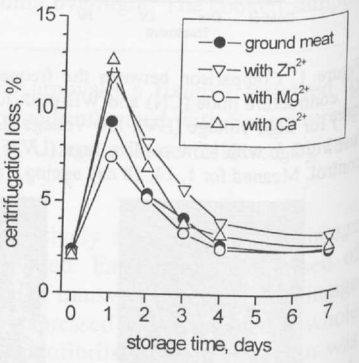



**Figure 1** Centrifugation loss from ground samples incubated with various divalent ions.

## Significance of the work

This experiment shows that slower degradation of some cytoskeletal proteins are associated with a poorer WHC in samples of porcine meat. This is in accordance with the hypothesis that intact intermediate filaments mediate the transport of water out of muscle fibres post-mortem, thereby playing a significant role in the development of drip loss.

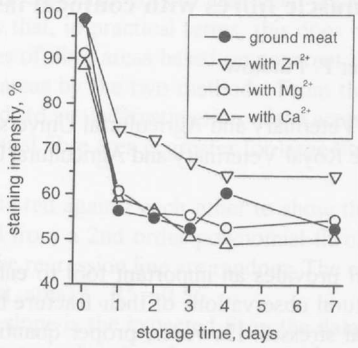
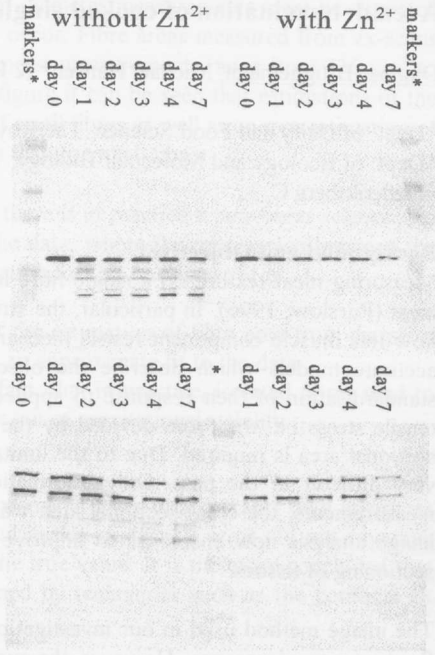



**Figure 3** Decrease in desmin staining intensity on western blots of ground samples.




* 205, 116, 82 and 47 kDa

**Figure 2** Western blots of desmin from samples stored in buffers with or without $Zn^{2+}$ (top) and vinculin from ground samples with or without added $Zn^{2+}$ (bottom).

## References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2. Morrison, E.H., Mielche, M.M. and Purslow, P.P. Immunolocalisation of intermediate filament proteins in porcine meat. Fibre-type and muscle-specific variations during conditioning. *Meat Science*, in press.