THE INFLUENCE OF INTRACELLULAR OSMOLARITY ON *RIGOR MORTIS*

Erik Slinde, Bjørn Roth, Jorma Suontama, Stine Balevik, Lars Stien and Anders Kissling.
Institute of Marine Research, Department of Aquaculture, P.O.Box 1870 Nordnes, N-5817, Bergen, Norway.
Phone: int+47-55236374, fax int+47-55236379, erik@imr.no.

Background

Our understanding of the biochemical reactions *post mortem* and how these affect the macroscopic quality in beef (Devine *et al.*, 1999) and salmon (Erikson, 2001) are not fully understood. The use of rigormeters that measure muscle tension and shortening continuously at different chilling rates and temperatures, make it possible to obtain conditions comparable to those under commercial conditions (Rosenvold, *et al.*, 2003). During our studies of tension and contraction in beef muscle (Rosenvold *et al.*, 2003) and stunning methods on *rigor mortis* in Atlantic salmon (Roth *et al.*, 2002) we have observed that the development of *rigor mortis* does not follow the tension and contraction curves in beef and the development of *rigor* index in Atlantic salmon. It has been pointed out that the mechanism of *rigor mortis* is not fully understood in fish (Erikson, 2001), and this is also the case for other animals such as beef (Devine *et al.*, 1999).

Objectives

The purpose of the present study has been to find other factors in the cell that contribute to the stiffness of *rigor mortis* beside the formation of the actomyosin bridges. Since *rigor mortis* is a general phenomenon in fish, birds and mammals, the findings have to be of general value.

Methods

Materials. Atlantic salmon (*Salmo salar*) was stunned and the gill arches were cut. Samples were taken from the region behind the dorsal fin. The *M. longissimus dorsi* fillets were from Norwegian red bulls. *Tension and contraction*. Rigormeters were from RigoTech®, Reologica Instruments AB, Lund, Sweden, and measure continuously tension and shortening/contraction in rectangular muscle strips 30 mm long and 10 x 10 mm cross sectional area. Calibration of the instruments using polyacylamide gels gave reproducible results (Rosenvold *et al.,* 2003). *Shear force.* WB shear force was measured using a Stable Micro Systems Texture Analyser, Godalming, UK. *Staining.* PAS, periodic acid Schiff method, was used to observe cell membranes and connective tissue (Carson, 1996). Serial sections, 12 µm thick, were cut on a cryostat and stained for glycogen. *Rigor index.* This was calculated as $I_R = (L_0 - L_t)/L_0 \times 100$ (Bito *et al.*, 1983) where L represents the vertical drop of the tail (part of fillet) when half of the length was outside the table. L_0 represent the vertical drop at start and L_t represents measurements during the experiment.

Results and Discussion

Since fish have a temperature that can be controlled at the moment of death they represent good models for the study of *rigor mortis*. Furthermore, in fish white and red muscle are separated and the pieces cut out are very homogenous. Figure 1 shows shortening and contraction in Atlantic salmon. Already within an hour after slaughter the tension and contraction have reached its maximum. In Figure 2, a number of shear forces and *rigor* indexes have been measured at comparable conditions. This figure shows that there is a tendency towards a decrease in shear force form the moment of death and onwards, while the *rigor* index goes through a maximum. *Rigor* index in fish are measured as tail bending (Figure 2), and a similar experiment has been performed on bovine *M. longissimus dorsi* (Figure 3 A and B) after wrapping in polyethylene film in order to restrict contraction (Hildrum *et al.*, 2002). After *rigor* the beef muscle relax, and becomes tender after 14 days as measured by WB. It is generally agreed that *rigor mortis* stiffness is due to the reaction between actin and myosin, and that this binding cannot be resolved due to lack of ATP. Both in fish and mammals the relaxation comes sooner than the tenderisation, thus something else must explain the stiffness.

After death catabolic reactions predominates. In general there is an increase in small molecules, for example glycogen goes to lactate. This increase the osmotic pressure ($\pi = cRT$) within a cell and water flows into the cell. The volume of both fish and wrapped fillets does not change during *rigor*. But due to change in osmotic pressure the shape of the muscle cell change from spherical to a more cubic form and the histological sections, as shown in Figure 4, change from a circular to a more squared form, and this has been calculated to be statistically significant. The WB shear force acting similar to a knife are not affected by the water being intra- or extracellular, as opposed to the *rigor* index and other methods used to measure texture during the *rigor mortis* phase (Erikson, 2001).

Conclusions

The *post mortem* reactions increase the number of molecules within the muscle cell. This increases the osmotic pressure within the cell and the cell is enlarged due to an osmotic influx of water. Since the volume of the muscle as such is constant the increased cell volume makes the muscle stiff. This explains why decrease in ATP, tension and contraction do not follow the direct measurement of muscle stiffness, *i.e. rigor mortis*. This is a general phenomenon for all animals. Better understanding of this water transport will increase our understanding of the water holding capacity of muscle food, as well as phenomenon's such as the PSE syndrome.

References

Bito, M., Yamada, K., Mikumo, Y. and Amano, K (1983) Differences in the mode of rigor mortis among some varieties of the fish by modifying Cutting`s method. Bull. Tokai Reg. Fish Res. Lab., 109, 89-93.

Carson, F.L. (1996) Histology 2end edition, ASCP Press, American Society of Clinical Patologists.

Devine, C.E., Wahlgren, N.M., and Tornberg, E. (1999) Effect of rigor temperature on muscle shortening and tenderisation of restrained and unrestrained beef *m. longissimus thoracisus et lumborum*. Meat Scie., 51, 61-72.

Erikson, U. (2001) Rigor measurements. *In*: Farmed fish quality. (*Ed.* Kerstin, S.C. and Warris P.D.) Blackwell Sci., London, pp.283-297.

Hildrum, K.I., Nilsen, B.N. and Wahlgren, N.M. (2002) Improving the tenderness of hot boned beef muscles. Fleiswirtschaft Int., 1, 42-48.

Rosenvold, K. Andersen, H.J. Slinde, E. and Hildrum, K.I. (2003) Early *post mortem* muscle shortening and tension in relation to tenderness in beef *M. longissimus*. J. Muscle Foods. Accepted.

Roth, B., Moeller, D., Veland, J.O. and Slinde, E. (2002) The effect of stunning methods on *rigor mortis* and texture properties of Atlantic salmon (*Salmo salar*). J. Food Sci. 67,1462-1466.

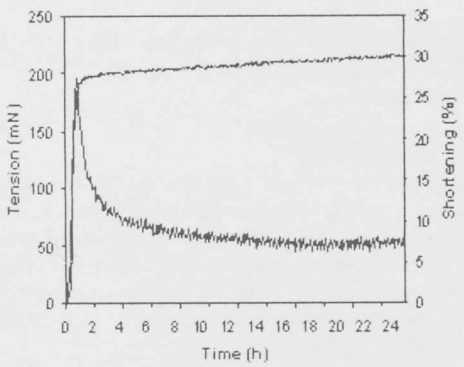

Figure 1. Isometric tension and isotonic contraction as a function of time for Atlantic salmon. The highly stressed fish was 4,7 kg and reared at 12 °C and measured at 20° C in the RigoTech®

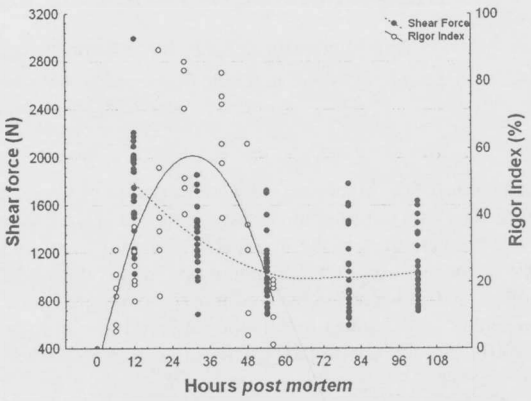

Figure 2. Measurments of WB shear force and *rigor* index in a number of different Atlantic salmon samples

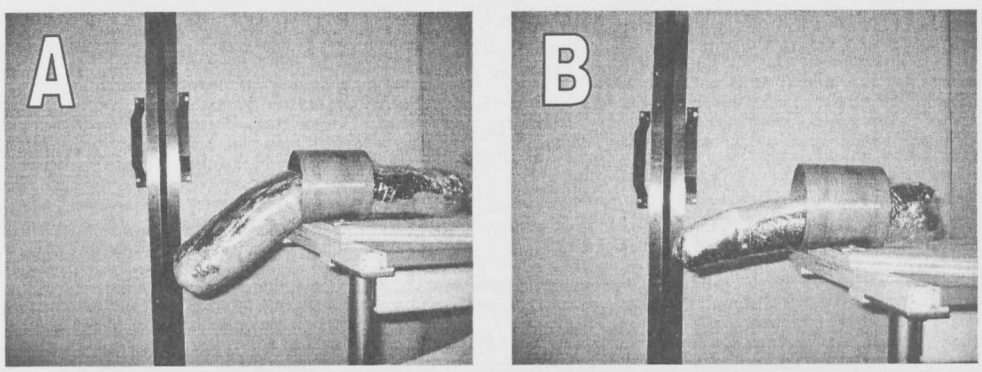

Figure 3 A and B. "Tailbending" of bovine *M. longissimus dorsi* being wrapped in plastic.

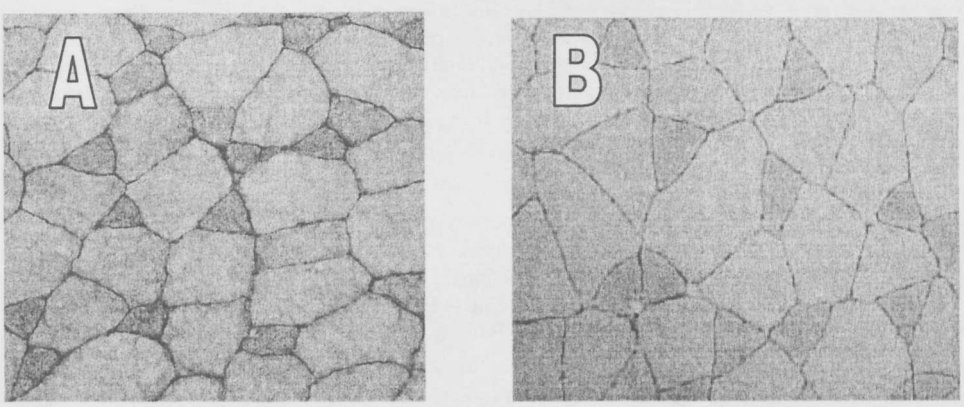

Figure 4. PAS reaction for glycogen in white muscle of an Atlantic salmon 3.2 kg, temperature 9 °C at harvest. (A) *Pre rigor* (after 30 min) and (B) *in rigor* (stored at 20 ° for 11 hours).