# THE INFLUENCE OF POSTMORTEM CHANGES IN BOVINE MUSCLE ON THE WATER-HOLDING CAPACITY OF BEEF. I.POSTMORTEM STORAGE OF MUSCLE AT 20°C

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## INTRODUCTION

The high water-holding capacity (WHC) of prerigor beef can be preserved by immediate salting and storage under refrigeration or by rapid freezing of the minced muscle, either unsalted or salted. Frankfurter or bologna type sausages made from such meat are of excellent quality (Hamm,1971,1978). For this type or prerigor processing a detailed knowledge on the dependence of WHC of beef on ATP concentration and tissue pH is important. It is, however, not yet clear in which way the changes in ATP and pH and the development of rigor mortis are related to the postmortem changes of WHC. It is also not yet known if the influence of these postmortem factors is different in the intact tissue, the unsalted and salted homogenates, which present the conditions pertaining in sausage mixtures. The experiments presented in this paper attempt to answer these questions.

## MATERIALS AND METHODS

Material. Beef neck muscles were obtained within 30-40 min postmortem (p.m.), portioned in 200 to 300 g pieces of about 1 cm thickness, sealed in polypropylene pouches, and stored at 20°C. After the required time of postmortem storage, one of the bags was randomly taken and a part of the sample ground through an electrical mincer using a plate with 4.5 mm holes. About 50-60 g were left as intact muscle. Unsalted and salted muscle homogenates were prepared at different times p.m. using 66 g of ground muscle, 33 g ice water or 33 g of an icecold 6 percent NaCl solution.

pH of tissue. 5 g of the intact tissue was homogenized with 20 ml dist. water and the pH measured using a combined glass electrode.

R-value and ATP determination. The determination of R-value, which is a fast method to detect the IMP/ATP ratio by measuring the optical density of a perchloric acid extract of the tissue at 250 and 260 nm and which allows an approximate estimation of the ATP content, was carried out using the procedure of Honikel and Fischer (1977). ATP was determined by the method of Jaworek et al. (1970).

Sarcomere length was measured according to the method of Voyle (1971) using a laser technique.

Extensibility of muscle. Neck muscles were taken from the carcass within 30 min p.m., cut longitidinally into sections of the same length and similar diameter (3-4 cm) and incubated at 21°C. At different times p.m. the muscles were stretched by loading with 2.3 kg, and the increase in length was measured. After release of the load the muscles were returned to 21°C.

Protein solubility. The results for the changes in solubility of myofibrillar proteins p.m. were taken from the work of Hamm and Grabowska (1979).

DSC studies. Differential scanning calorimetry was performed using the Perkin-Elmer DSC-II. Weighed samples of muscle (38-42 mg) were sealed in large-volume capsules. Water was used as reference. The experiments were conducted with a heating rate of 10 K/min over the temperature range 275-370 K.

Water-holding capacity (cooking loss). About 5 g of the intact muscle or of the unsalted (immediately after homogenizing) or salted muscle homogenates (after 30 min equilibration) were weighed into a preweighed centrifuge tube. The tube, covered with a glass marble, was heated in a boiling water bath for 20 min. After cooling, the juice released was drained, the meat sample blotted with filter paper and the cooking loss determined (Yu Beng Lee et al., 1978).

## RESULTS AND DISCUSSION

The rigor mortis of bovine neck muscles, as defined by the decrease of extensibility of muscle fibers, occurred when the postmortem pH decreased to pH values around 5.9 and the R value had reached a level around 1.10 (Fig.1) which corresponds to an ATP concentration of about 1.0 µMol/g tissue (Honikel and Fischer,1977; see also insert in Fig. 3A). Neither the loaded nor the unloaded muscle shortened before these values were reached. This observation is supported by the measurements of sarcomere length (Fig.2). The shortening of sarcomeres might be caused by gliding of myofilaments whereas the decrease of extensibility will be primarily due to cross-linking between filaments. From these results it can be concluded that the changes of pH p.m. before the onset of rigor mortis at 20°C are not associated with a shortening of muscle and that a certain shortening occurs at the onset of rigor. In the prerigor state, the activity of the calcium pump of the sarcoplasmic reticulum at 20°C keeps the $Ca^{2+}$ concentration around the myofilaments very low (Honikel and Hamm,1978) and,therefore, prevents contraction. However, if the ATP level falls below about 1 µMol/g, $Ca^{2+}$ is released from the SR and initiates shortening as well as rigor.

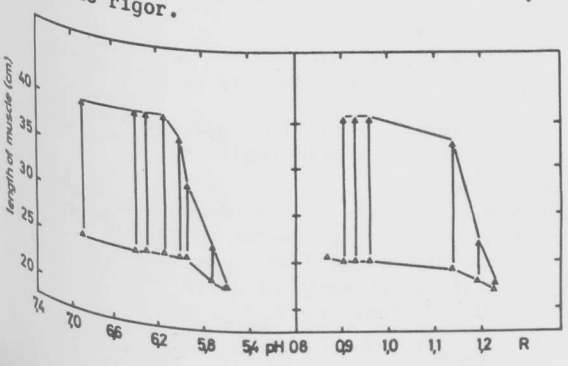


Fig.1 Extensibility of M.mastoideus of beef in relation to postmortem changes of pH and R-value.
Temperature was 21°C; during the storage periods the muscle was wrapped in aluminum foil to avoid surface drying. The experiments were repeated 3 times with similar results.
(△) length of muscle without load;
(▲) length of muscle under load; the vertical lines indicate the extensibility.

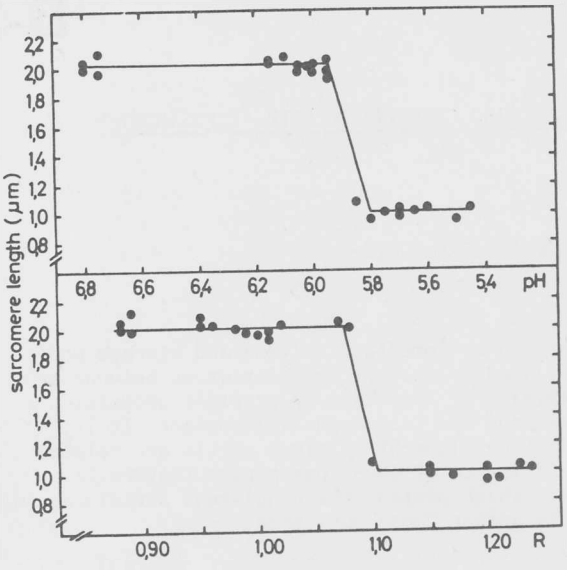


Fig. 2 Relation between rigor shortening and postmortem pH and R-value in bovine neck mucles at 30°C. The prerigor muscles were incubated at 30°C in plastic pouches for different times p.m.

Rigor mortis and protein changes

In the same pH range, in which rigor mortis occurs (around pH 6) a remarkable decrease of the solubility of myofibrillar proteins could be observed (Fig. 3A). This postmortem change of protein solubility, which has been reported by many authors (for references see Hamm and Grabowska,1979), is almost certainly due to the strong association of myosin and actin after the depletion of ATP.

Some loss of solubility had already occurred before the onset of rigor but the change in solubility per pH unit was much smaller in the prerigor phase than during the development of rigor (Fig. 3A). The decrease of solubility in the prerigor phase (above pH 6.1) parallels the pH dependence of solubility of rigor muscle which was studied by adjusting rigor muscle homogenates to prerigor pH values (Fig. 3A, lower curve). From these results it can be concluded that the decrease in protein solubility in the prerigor phase is caused by the fall of pH only and not by the breakdown of ATP. The pronounced loss of solubility below pH 6.1, however, must primarily be caused (at least two thirds) by the development of rigor mortis.

The change in the solubility of myofibrillar proteins during the development of rigor might be due to conformational changes of these proteins; this is supported by the results of DSC studies. Considerable postmortem changes in the exothermal peak of muscle tissue at 53-55°C could be observed (Fig. 4). In studies, which are not reported here, we showed that the decrease of this exothermal peak was not caused by the breakdown of ATP but seemed to be the result of conformational changes in muscle proteins, which occur at the onset of rigor mortis (pH 5.9, R 1.05). There are apparently no conformational changes during the prerigor state. These findings are in agreement with the measurements of muscle extensibility and muscle shortening (Fig.1 and 2).

Postmortem changes and water-holding capacity

The development of rigor mortis in the intact muscle had no significant effect on the WHC of unsalted muscle homogenates prepared at different times p.m., because the cooking loss decreased slightly and continuously with the postmortem fall of pH (Fig. 5). Development of rigor in the intact muscle, however, exerted a substantial effect on WHC of salted muscle homogenates. At the onset of rigor, i.e. after reaching pH 5.9 (Fig. 5 and 3B) and R = 1.05 (Fig. 6) a considerable increase of cooking loss i.e. a strong decrease of WHC occurred.

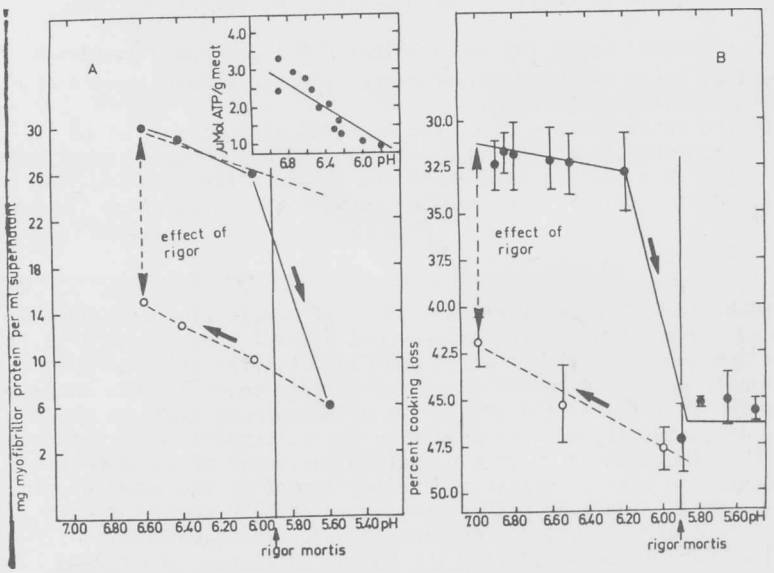


Fig. 3
Changes of soluble myofibrillar protein and cooking loss of salted homogenates in relation to pH.
(A) Bovine neck muscles incubated at 20°C were ground at certain pH values, homogenates with 2 percent salt were prepared and the amount of soluble myofibrillar proteins determined (as described by Grabowska and Hamm, 1979)(●). After the final pH in the intact muscle was reached homogenates were prepared in the same way, the pH was readjusted with 1 M NaOH and the amount of soluble myofibrillar protein was determined (o).
(B) The homogenates were prepared as in (A) and the percent cooking loss was determined. Bars indicate standard deviation of 4 experiments.
(Insert in A). The insert shows the relationship between ATP concentration and pH p.m. in bovine neck muscles incubated at 20°C.

These results are in agreement with those of Jolley et al. (1980) obtained by studying the effect of rigor mortis on WHC of raw, bovine neck muscle and raw salted muscle homogenates using a filter-paper press-method.

Addition of salt to the tissue homogenates caused an increase of WHC (decrease of cooking loss) which was much more pronounced prerigor than after the onset of rigor (Fig. 5). This known fact can be explained by the electostatic theory of swelling (Hamm,1960,1972).

The result of the experiment presented in Fig. 3B shows that the change in WHC per pH unit obtained by the pH alteration of rigor muscle homogenate (lower curve) is not smaller than that observed during pH fall prerigor (upper curve in Fig. 3B). From these results it can be concluded that the rather small decrease of WHC of salted or unsalted muscle homogenates, in the prerigor phase is caused by the fall of pH only. At least two thirds of the appreciable loss of WHC of the salted homogenates between pH 6.2 and 5.9, however must be due to the development of rigor mortis.

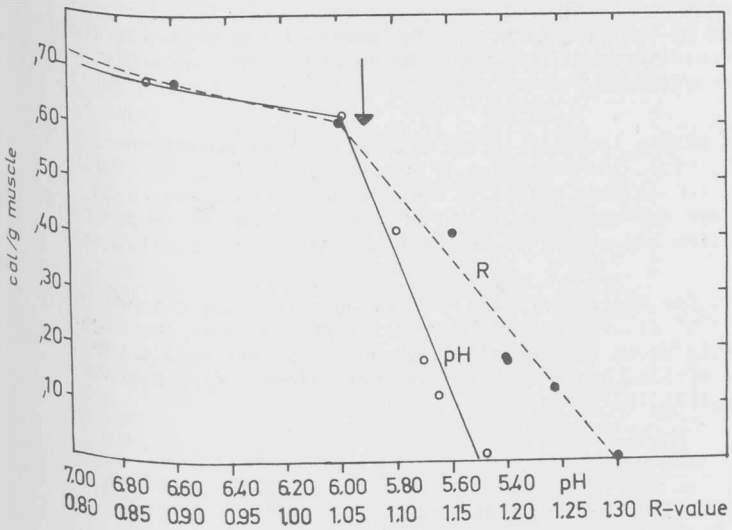


Fig. 4

Area of the exothermal heat output in the DSC thermogram with the peak at about 327 K of bovine neck muscles in relation to pH and R value.

The arrow indicates the onset of rigor mortis (pH 5.9 and R around 1.08).

The similar pattern of the curves in Fig. 3A and 3B suggests a close relationship between the change in solubility of myofibrillar proteins, induced by postmortem metabolism, and the WHC of salted tissue homogenates. Such a relationship was extensively discussed by Hamm and Grabowska (1979).

In our experiments with salted muscle homogenates no direct correlation between the change in ATP concentration p.m. and WHC existed (see Fig. 6 and insert in Fig. 3A). The large fall of WHC (increase of cooking loss) starting at about pH 5.9 and R = 1.05 shows that it is not the postmortem ATP hydrolysis itself but the development of rigor mortis, initiated by the depletion of ATP, which is the main reason for postmortem alteration of WHC of salted muscle homogenates, prepared at different times p.m. The decrease of WHC in the prerigor phase is apparently not related to the breakdown of ATP but to the fall of pH caused by glycolysis.

The question arises as to why the development of rigor has an effect on the WHC of salted muscle homogenates rather than on the WHC of unsalted homogenates (Fig. 5) or of unsalted intact muscle (Jolley et al. 1980). By the completion of the postmortem drop of pH from 7 to 5.9, at which the onset of rigor occurs, the myofibrillar protein is approaching the IP; this means an increase of oppositely charged groups and, therefore, an increase of intermolecular ionogenic cross-linkages. Less water can be immobilized in the network of myofibrillar

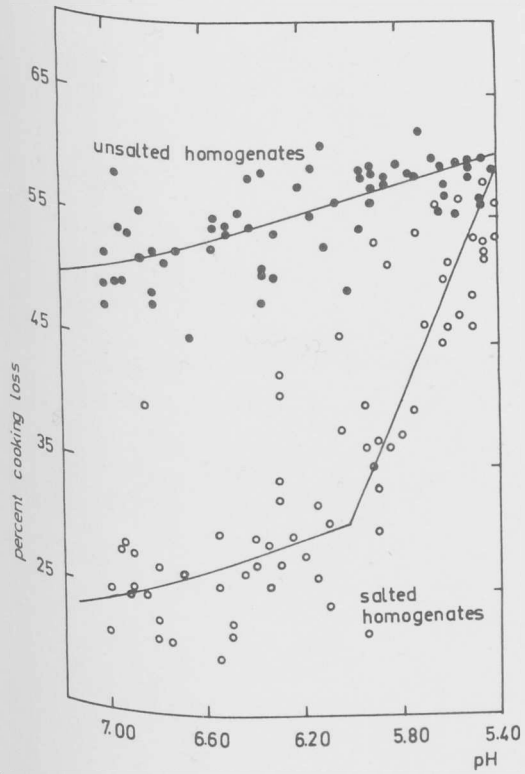


Fig. 5 Relationship between percent cooking loss of unsalted and salted muscle homogenates and pH of the corresponding bovine neck muscles. The pH of the intact muscles was measured and homogenates were prepared and cooking loss measured. The samples were taken from 21 different animals.

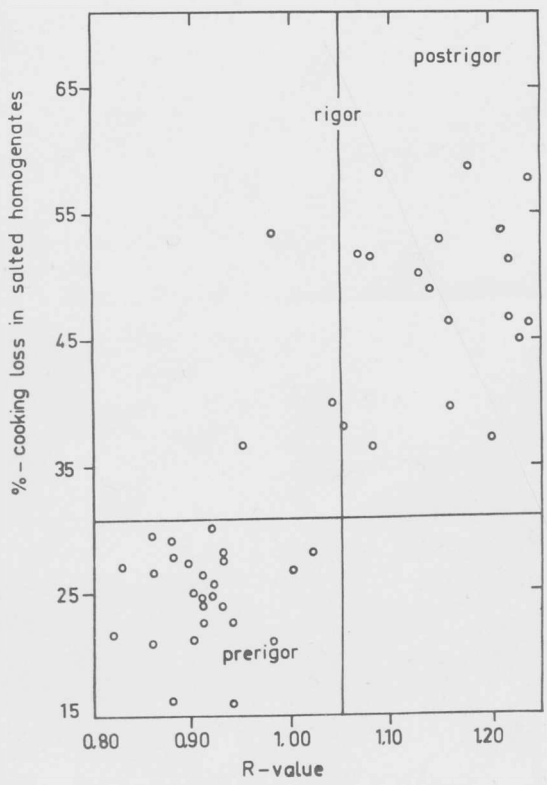


Fig. 6 Cooking loss in salted homogenates and R -value in the corresponding muscle pieces.

proteins, tightened in this way, than in the loose network existing at higher pH; the result is a decrease of WHC according to the general concept of the influence of protein charges on WHC and swelling of muscle (Hamm, 1960,1972,1975). This pH dependent type of intermolecular cross-linking must be so strong that an additional cross-linking between myofilaments caused by rigor development, cannot exert an additional effect on WHC in the absence of salt.

Addition of salt at a pH higher than the IP causes a strong increase in WHC and swelling of muscle because of a shift of the IP to lower pH values (Hamm,1960,1972,1975). The formation of interfilamental cross-linking during rigor will hinder this effect. Therefore, the WHC-raising effect of NaCl in muscle homogenates is diminished with proceeding development of rigor. Before the onset of rigor, pH dependent changes of protein charges determine the postmortem alteration of unsalted and of salted muscle homogenates to a similar extent but at a different level (Fig. 5).

The results of this paper lead to the conclusion that for the preparation of sausages from hot-deboned beef the meat should be minced and salted before the onset of rigor mortis. The actual time p.m. when the rigor occurs depends on the temperature of conditioning as is shown in the following paper (Honikel et al. 1980). In beef, minced and salted before the onset of rigor mortis, a high WHC is also maintained during storage under refrigeration, freezing or freeze-dehydration (Hamm,1972,1977,1978).

REFERENCES


Hamm,R. 1960: Advanc. Food Research 10, 355.
Hamm,R. 1972: "Kolloidchemie des Fleisches". P.Parey Verlag, Berlin, Hamburg.
Hamm,R. 1975: In "Meat" (Ed. J.A.Cole, R.A. Lawrie).Butterworths, London, p.321.
Hamm,R. 1977: Meat Sci. 1, 15.
Hamm,R. 1978: Proceed. Meat Ind. Confer. AMSA, AMIF. p. 32.
Hamm,R. and Grabowska, J. 1979: Fleischwirtschaft 59, 1138.
Honikel,K.O. and Fischer, C. 1977: J. Food Sci. 42, 1633.
Honikel, K.O. and Hamm,R. 1978: Meat Sci. 2, 181.
Honikel, K.O., Hamid,A., Fischer,C. and Hamm,R. 1980: Proceed. 26th Europ.Meat Research Worker's Meeting, Colorado Springs.
Jaworek,D., Gruber,W. and Bergmeyer,H.U. 1970: In "Methoden der enzymatischen Analyse" 2. Ed. (Edit.H.U.Bergmeyer). Verlag Chemie, Weinheim, p. 2020.
Jolley,P.H., Honikel,K.O. and Hamm,R. 1980: Meat Sci. in press.
Voyle,C.A. 1971: Proceed. 17th Europ. Meat Research Worker's Meeting. Bristol, p. 95.
Yu Beng Lee, Rikansrud,D.A., Hagberg,E.C. and Forsythe, R.H. 1978: J. Food Sci. 43, 35.