INFLUENCE OF COOLING AND FREEZING OF MINCED PRE-RIGOR MUSCLE ON THE BREAKDOWN OF ATP AND GLYCOGEN

K. O. HONIKEL & R. HAMM

Department of Chemistry and Physics, Federal Institute of Meat Research, Kulmbach, Federal Republic of Germany

(Received: 28 July, 1977)

SUMMARY

The high water-holding capacity of pre-rigor beef can be preserved for months by rapid freezing of the minced salted or unsalted bovine muscle before onset of the breakdown of ATP. If beef frozen in this way is processed without preceding thawing, sausages of excellent quality are obtained. The same result can be obtained using beef salted and freeze-dried in the pre-rigor state. It is important in both procedures to ensure that the depletion of ATP during freezing is kept to a minimum. The optimum conditions for cooling and freezing were therefore studied.

If NaCl is not added, the rate of ATP breakdown decreases with falling temperature to about +6°C but then increases with further cooling, reaching a maximum rate at about −1°C, at which temperature the meat remains for a relatively long period during freezing. If the beef is salted, the rate of ATP hydrolysis decreases with falling temperature until the meat is frozen. Above +3°C the concentration of ATP is lower in salted than unsalted meat in the first hours post mortem, but below this temperature the position is reversed. The influence of temperature on lactate formation, i.e. on the rate of glycolysis in the presence and absence of salt, follows similar patterns. Therefore, it is better to salt the beef before freezing rather than during the preparation of the sausage emulsion. These influences of temperature on the ATP depletion in unsalted and salted beef can be explained in terms of the release of Ca^{2+} ions from the sarcoplasmic reticulum.

INTRODUCTION

Muscle in the pre-rigor state has a higher water-holding capacity (WHC) than muscle in the rigor or post-rigor states and therefore produces sausages with reduced moisture loss and less rendering out of fat when cooked (Hamm, 1972, 1973). These superior processing properties of pre-rigor muscle are directly related to its high

Meat Science (2) (1978)—
Printed in Great Britain

level of adenosine triphosphate (ATP). The high WHC of pre-rigor beef can be preserved for months by rapid freezing of the minced bovine muscle, either salted or unsalted, before onset of the enzymatic breakdown of ATP. During frozen storage at temperatures of −20 °C or lower the ATP concentration remains almost unchanged. If beef frozen in this way is processed without preceding thawing, sausages of excellent quality are obtained (Hamm, 1972). The same result can be achieved using minced beef salted and freeze-dried in the pre-rigor state and then rehydrated (Hamm & Potthast, 1975; Hamm, 1977*a*). In both types of procedure it is important that the breakdown of ATP during freezing is kept to a minimum. For this reason the optimum conditions of cooling and freezing of minced pre-rigor beef for use in sausage production were studied. For the purpose of comparison freezing of non-minced pre-rigor muscle was also included in these studies.

MATERIAL AND METHODS

Samples from the *sternomandibularis* muscle of bull carcases were taken—from a commercial abattoir—immediately after slaughter. For each temperature studied a muscle slice (150 g), a 50 g sample of minced muscle (4·5 mm plate) and a 50 g sample of minced and salted (2 % NaCl) muscle were sealed in polypropylene pouches. Both sliced and minced beef had a thickness of 1 cm. The pouches were immersed into the bath of a cryostat, which was adjusted to the particular temperature (20–30 min post mortem). After 10–20 min the centre of the sample had reached the bath temperature (zero time). After a time of immersion of 3 h or 3·5 h the samples were analysed.

Lactate, which was also determined in the fresh sample before immersion in the cryostat bath, was enzymically determined after Hohorst (1970). The ATP breakdown was followed by means of the 'R-value' after the method of Honikel & Fischer (1977). This photometric procedure, in which the ratio of absorbance at 250 and 260 nm is measured, presents the extent of the enzymatic transformation of ATP into inosine derivatives post mortem. The R-value is highly correlated with the ATP concentration (Honikel, 1976); the higher the R-value, the lower the ATP concentration. After a complete disappearance of ATP the R value can still increase somewhat because of the breakdown of ADP and AMP present at the beginning of the experiment.

For each temperature investigated the muscle samples were taken from another animal.

RESULTS

Figure 1 shows the influence of temperature on the extent of transformation of ATP into inosine monophosphate (IMP) (R-value) 3·5 h post mortem. These figures

indicate the temperature effect on the rate of the breakdown of ATP. The influence of temperature on the lactate formation within 3 h post mortem, i.e. on the rate of glycolysis, is shown in Fig. 2. In muscle slices, the influence of temperature on the rate of ATP breakdown is relatively small; there is a slight decrease between +24° and +6 °C. With further lowering of temperature the rate of ATP breakdown increases, reaching a small maximum at about −1 °C (Fig. 1).

The influence of temperature on the lactate formation post mortem follows the same pattern but the changes are much more pronounced (Fig. 2). Here a sharp maximum of lactate formation at −1 °C was observed. From the curves in Figs. 1 and 2 it can be concluded that lowering the temperature from +5 °C to −1 °C causes a strong increase in the turnover of ATP in the non-minced tissue because ATP turnover = 2Δ[ATP] + 1·5Δ[Lactate].

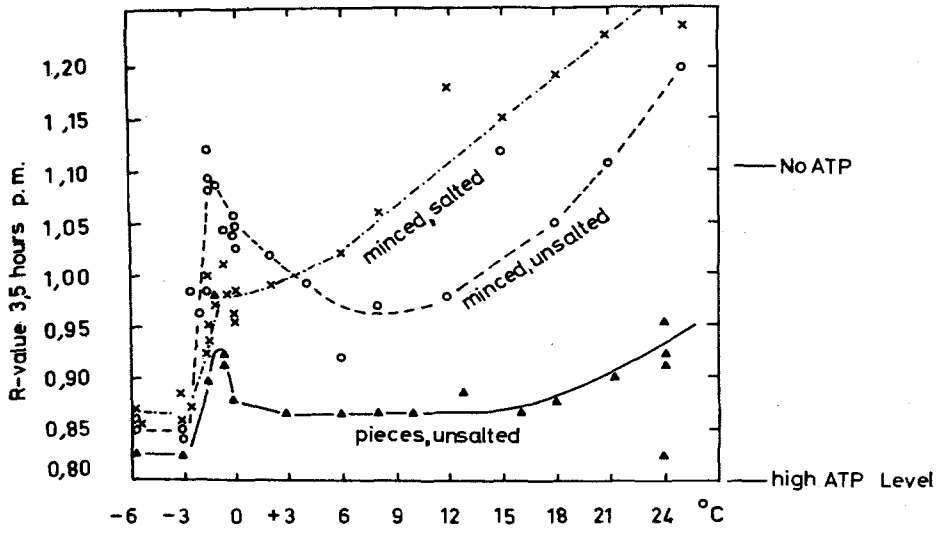

Fig. 1. Influence of temperature on the extent of the postmortem transformation of ATP to IMP ('R-value') in bovine *sternomandibularis* muscle under different conditions.

The effect of temperature on the postmortem changes in the minced, unsalted muscle samples follows a pattern similar to that of the non-minced tissue. The maximum rate of ATP breakdown at about −1 °C, however, is much more pronounced (Figs. 1 and 3) whereas the maximum of lactate formation at this temperature is less marked (Figs. 2 and 3). From these results it can be concluded that between +6 °C and −1 °C the turnover of ATP also increases in the minced tissue.

The influence of temperature on the postmortem changes in the minced and salted

samples shows a completely different pattern. In this case the rates of ATP breakdown and lactate formation decrease continuously with decreasing temperature until the tissue is frozen; no maxima at −1 °C were observed (Figs. 1 and 2).

In all samples, between −1 °C and −6 °C, i.e. at freezing temperatures, the rates of ATP breakdown and glycolysis (lactate formation) decrease strongly with falling temperature. Maximum rates of ATP depletion and lactate accumulation at −3 °C, as they were observed by Behnke *et al.* (1973) in the intact bovine *sternomandibularis* muscle, were not found in our experiments.

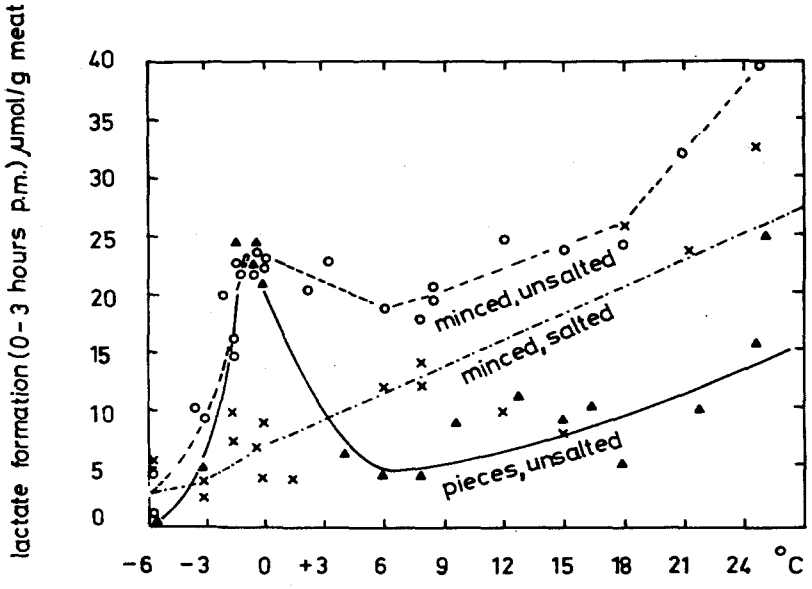

Fig. 2. Influence of temperature on the postmortem lactate formation in bovine *sternomandibularis* muscle under different conditions.

In order to elucidate the role of calcium ions, released from the sarcoplasmic reticulum (see section below headed 'Discussion'), on the effect of temperature on the biochemical postmortem changes in unsalted minced bovine muscle, 15 µmoles of the calcium sequestering agent EGTA (ethylene-glycol-bis(aminoethyl-ether)-N,N'-tetraacetic acid) were added to 1 g homogenates of pre-rigor muscle. The homogenates were then incubated at different temperatures between −1 °C and +14 °C. As Fig. 3 shows, in the presence of EGTA the rates of lactate formation, pH fall and ATP depletion decrease continuously with falling temperature without the rate maxima at −1 °C observed in the control samples.

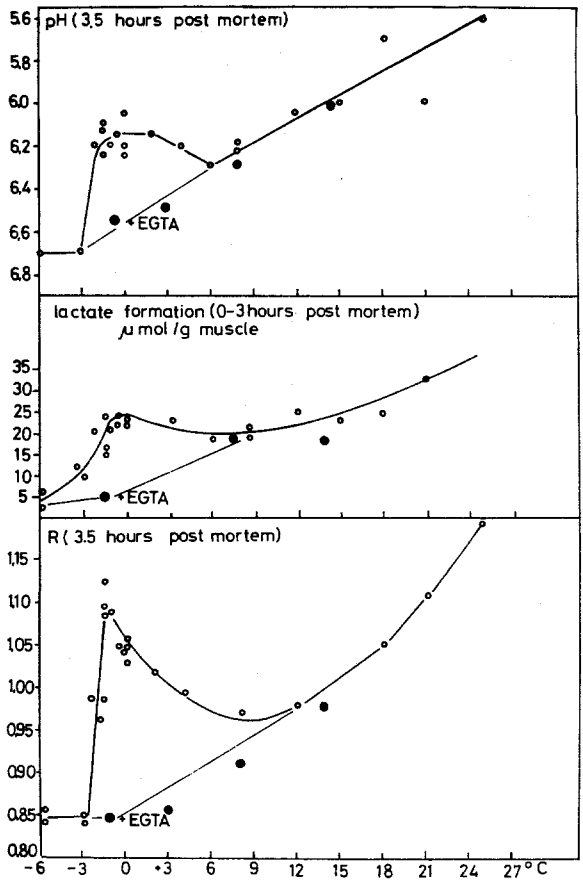

Fig. 3. Effect of temperature on biochemical postmortem changes in a homogenate of bovine *sternomandibularis* muscle as influenced by the addition of EGTA (15 µmoles/g homogenate).

DISCUSSION

The decrease in the rates of ATP and glycogen breakdown post mortem by lowering the temperature from + 24 °C to about + 6 °C as observed in all samples is due to the normal influence of temperature on enzymic reactions. The increase in the rate of ATP turnover between + 6 °C and − 1 °C in the intact tissue (muscle slices) is well known to be associated with the 'cold shortening' phenomenon, i.e. the toughening of meat by rapid cooling of pre-rigor meat (see Bendall, 1973; Honikel, 1975;

Hamm, 1976). However, according to the results reported in the literature, the minimum rate of ATP turnover should be at about +15 °C whereas, in our experiments, it was at about +6 °C. The finding of Bendall (1973) that during 'cold shortening' the ATP level remains rather constant because of a fast resynthesis of ATP from adenosine diphosphate by the glycolytic process is confirmed by our results.

The 'cold shortening' phenomenon is explained by the influence of temperature on the membrane system of the sarcoplasmic reticulum (SR). Below about +15 °C decreasing temperature causes an increasing inactivation of the ATP-driven calcium pump of the SR, which transports Ca^{2+} ions from the sarcoplasma into the SR. Therefore, Ca^{2+} ions are released from the sarcotubular system; they activate the myosin adenosine triphosphatase (ATPase) and, consequently, initiate the onset of rigor mortis (Bendall, 1973). It is not yet clear whether temperature effects on mitochondrial membranes also participate in the release of Ca^{2+} ions.

Surprisingly, with minced muscle also an increase in ATP turnover between +6 °C and −1 °C was observed (Figs. 1 and 2). Mincing causes an acceleration of the breakdown of ATP and glycogen; this might be due to an activation of myosin ATPase by the release of calcium ions from the SR which is partially damaged by the process of mincing (Hamm & van Hoof, 1971; Hamm, 1976). The marked maximum of the rate of ATP breakdown at −1 °C suggests that at higher temperatures the calcium pump of the SR is effective also in the minced tissue. It should be mentioned that the Ca^{2+} transport mechanism (Martonosi, 1975) is also exerted by SR fragments (microsomes). Therefore, fragmentation of the SR by mincing the muscle might not completely destroy the Ca^{2+} accumulating capacity. In contrast to the intact muscle, the ATP disappears very rapidly at −1 °C because most of the glycogen has already been metabolised in the minced muscle three hours post mortem (Hamm & van Hoof, 1971) and so further resynthesis of ATP cannot occur. It could be that the high rate of ATP breakdown just at the freezing temperature of tissue (~ -1 °C) causes the strong activation of phosphofructokinase which Dalrymple & Hamm (1975) observed during freezing of pre-rigor minced bovine and rabbit muscles.

At temperatures $\geqq 3$ °C salting of the minced muscle causes an increase in the rate of ATP depletion (Fig. 1). This had already been observed by Van Hoof & Hamm (1973) and by Hamm (1977*b*) and explained by an exchange of Ca^{2+} ions from the SR against Na^{+} ions of the NaCl. The Ca^{2+} so released accelerates the ATP breakdown by additional activation of myosin ATPase. If most of the Ca^{2+} present in the SR fragments is released by this exchange mechanism, lowering the temperature cannot cause an additional release of Ca^{2+} and, consequently, no increase in the rate of ATP depletion. This corresponds exactly to our observation that with salted minced muscle there is no maximum of ATP breakdown at −1 °C. Lowering the temperature from +24 °C to the freezing point of tissue results in the normal continuous reduction in the rate of enzymatic postmortem reactions. The

observation that the rate of ATP breakdown after freezing and thawing of pre-rigor frozen bovine muscle is slower at +3 °C than at +25 °C (Okubanjo & Stouffer, 1975), is in some agreement with our results obtained with salted beef because freezing and thawing also causes a release of Ca^{++} from the SR (Kushmerik & Davies, 1968).

The question arises as to why, between about +4 °C and the freezing of tissue (about −1 °C), the rate of ATP breakdown in the minced muscle is higher in the absence of NaCl than in the presence of salt. This phenomenon can be explained by the fact that increase in the ionic strength (by addition of monovalent ions) normally alters the physical and enzymic properties of proteins. Salting of muscle tissue in the rigor or post-rigor states inhibits the ATPase, as has been shown by Hamm & van Hoof (1974). Also, the production of lactate is retarded (Figs. 2 and 3) by salting (van Hoof & Hamm, 1973). It seems not unlikely, therefore, that the Ca^{2+} activation of the myosin ATPase is also altered by NaCl. In this case the Ca^{2+} ions released from the SR are unable to cause a normal stimulation of the ATP turnover.

Thus, in the minced and salted muscle, a continuous decrease in the ATP turnover with falling temperature occurs, as revealed by the decrease in the R-value (Fig. 1) and in the lactate formation (Fig. 2). In the unsalted minced tissue, however, the Ca^{2+} release from the SR at low temperatures stimulates the ATP turnover.

The observation that addition of salt lowers the rate of postmortem glycolysis even at temperatures > +6 °C confirms earlier results according to which the glycolysis in minced muscle is inhibited by NaCl (not at the beginning but in later stages) (van Hoof & Hamm, 1973); this is due to the simultaneous effect of low pH and elevated ionic strength which causes an inactivation of glycolytic enzymes (Dalrymple & Hamm, 1974).

As this discussion has shown, the increase in the rate of the breakdown of ATP and glycogen in minced, unsalted bovine muscles between +6 °C and −1 °C can be explained by a release of Ca^{2+} ions from the SR in this temperature range. Addition of a calcium-sequestering agent such as EGTA to a homogenate of pre-rigor bovine muscle at temperatures from −1 °C to +14 °C eliminates completely the maxima of the rates of ATP depletion and lactate formation and the pH minimum which were observed at this temperature in the absence of EGTA (Fig. 3). This result can be regarded as evidence for the postulated role of SR calcium in the phenomena discussed in this paper.

CONCLUSIONS

For sausage production, the minced pre-rigor beef should be frozen—or freeze-dehydrated—in such a way as to ensure that the breakdown of tissue ATP is kept to a minimum. As has been shown in this paper, if NaCl is not added, the rate of ATP breakdown decreases with falling temperature until about +6 °C but increases with

further cooling, reaching a maximum at about −1 °C at which temperature the meat remains for a relatively long period during the freezing process (Fennema *et al.*, 1973). If the minced beef is salted, the rate of ATP depletion decreases with falling temperature until the meat is frozen. In the critical temperature range around −1 °C, the ATP is disappearing faster in the salted than in the unsalted meat. Therefore, it is better to salt the pre-rigor meat before freezing than to add the salt during the preparation of the sausage emulsion. The influence of freezing on lactate formation by glycolysis follows a similar pattern. Around −1 °C glycolysis occurs much faster in the unsalted meat than in the salted. This means that the drop in pH is faster in the unsalted meat (Fig. 3). Lower pH values result in lower WHC of the beef (Hamm, 1972). Thus, with regard to this effect also, salting of pre-rigor beef before freezing is advantageous.

REFERENCES

BEHNKE, J. R., FENNEMA, O. R. & CASSENS, R. G. (1973). *J. agric. Chem.*, **21,** 5.
BENDALL, J. R. (1973). *Proc. 19th Eur. Meat Res. Wkrs., Paris*, **1,** 1.
DALRYMPLE, R. H. & HAMM, R. (1974). *Fleischwirts.*, **54,** 1084.
DALRYMPLE, R. H. & HAMM, R. (1975). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **158,** 333.
FENNEMA, O. R., POWRIE, W. D. & MARTH, E. H. (1973). *Low temperature preservation of food and living matter.* New York, M. Dekker Inc.
HAMM, R. (1972). *Kolloidchemie des Fleisches.* Berlin, Hamburg, Paul Parey Verlag.
HAMM, R. (1973). *Fleischwirts.*, **53,** 73.
HAMM, R. (1976). *Fleischwirts.*, **56,** 79.
HAMM, R. (1977*a*). *Fleischwirts.*, **57,** 1155.
HAMM, R. (1977*b*). *Meat Sci.*, **1,** 15.
HAMM, R. & POTTHAST, K. (1975). *Fleischwirts.*, **55,** 87.
HAMM, R. & VAN HOOF, J. (1971). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **147,** 193.
HAMM, R. & VAN HOOF, J. (1974). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **156,** 87.
HOHORST, H. H. (1970). In *Methoden der enzymatischen Analyse* (2nd ed.) Bergmeyer, H. U. (ed.) p. 1425, Weinyeim, Verlag Chemie.
HONIKEL, K. O. (1975). *Fleischwirts.*, **55,** 888.
HONIKEL, K. O. (1976). *Fleischwirts.*, **56,** 1163.
HONIKEL, K. O. & FISCHER, CH. (1977). *Fleischwirts.*, **57,** 1015.
KUSHMERIK, M. S. & DAVIES, R. E. (1968). *Biochim. biophys. Acta*, **153,** 279.
MARTONOSI, N. (1975). In *Calcium transport in contraction and secretion* (Carafoli, E., Clementi, F., Drabikowski, W. & Margreth, A. (eds.)) p. 31. Amsterdam, North Holland Publ. Co.
OKUBANJO, A. O. & STOUFFER, J. R. (1975). *J. Fd Sci.*, **40,** 955.
VAN HOOF, J. & HAMM, R. (1973). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **150,** 182.