The Influence of Temperature on Shortening and Rigor Onset in Beef Muscle

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At room temperature the onset of rigor mortis in a muscle is defined as the beginning of the loss of extensibility (Bendall, 1973a). At 20°C the rigor in normal bovine muscle (no DFD) is setting on at pH values around pH 5.9 (Currie and Wolfe, 1980; Honikel et al., 1981). This development of rigor is a slow process and it takes some time till the total loss of extensibility (completion of rigor) is reached (Bendall, 1973a; Currie and Wolfe, 1979). Besides the loss of extensibility and the development of rigor there is at room temperature and above also a limited shortening in unrestrained muscles (rigor shortening) occuring at approximately the same pH range as the rigor onset occurs. Furthermore at chilling temperatures cold shortening leads to muscle contracture. Shortening of a muscle in general and the development of rigor influence the tenderness and the water retention of beef. Locker (1960), Marsh and Leet (1966), Locker and Daines (1975) reported about a higher toughness in shortened muscle. Powell (1978), and Honikel et al. (1980) found higher drip losses in contracted than in unshortened muscles. The onset of rigor decreases the water-holding capacity (WHC) of salted homogenates and meat batters (Jolley et al., 1980/81; Honikel et al.1980,1981). These facts are of paramount importance for the handling and processing of freshly slaughtered (hot-boned) meat. Knowledge on the influence of time postmortem and conditioning temperature on the onset of rigor mortis will help to reduce drip loss in meat and to improve the WHC of salted meat products.

Material and Methods

Beef neck muscles (M.sternomandibularis and M. mastoideus) were excised from the carcass within 30 - 40 min after slaughter. Two corresponding muscles of both sides of an animal were cut to the same length and shape and incubated at temperatures between -1° and +38°C in a thermostate. For each temperature another pair of muscles was taken. The trimmed muscles were about 25 cm long and had a cross section area of 7 - 11 cm^2. One muscle of the pair was used for extensibility and shortening experiments the other one was used for the measurements of pH fall and R-value determinations during the experiment.

Determination of R-value

The determination of the R-value is a fast spectrophotometric method for the estimation of the degree of transformation of ATP to IMP and serves therefore as indicator of the ATP breakdown in postmortem muscle (Honikel and Fischer, 1977).

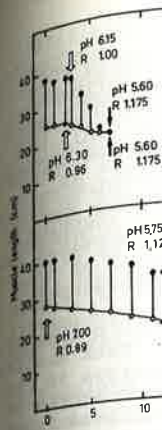
Extensibility of muscle

After different times of postmortem storage in one half of a muscle pair from a carcass the length of the unrestrained, unloaded muscle was measured (unloaded length), simultaneously with pH and R-value determination in the other half; then the muscle strip was hanged up and stretched by loading with 2.3 kg (about 250 g/cm^2 as a mean value) and the increase in length was measured (loaded length) for a short time. After taking off the load, the muscle returned to its initial length. The procedure of loading and unloading was repeated with the same muscle until all extensibility was lost. The extensibility is the difference in length of loaded minus unloaded muscle at each time, independent on the absolute values of the muscle length. The loss of extensibility is defined as the moment when the difference value of length becomes smaller than the previous measured value.

Results

1. Effect of storage temperature on muscle shortening and extensibility

The behaviour of muscles stored shortly after death at different temperatures within the temperature range of -1° and +38°C differs considerably. The differences are observed in the patterns of shortening and loss of extensibility and are related to time of storage or to the rate of pH decrease and ATP depletion. According to these variations we considered four distinct temperature groups. 1. The nearly physiological temperatures (34-38°C); 2. the range between 18-33°C; 3. the range between 5-17°C; and 4. the pre-freeze chilling temperatures (-1 till +4°C). For each of these groups a typical example of muscle shortening and extensibility behaviour is presented in Fig. 1. The moment of loss of extensibility or increase of resistance to stretch, which according to Bendall (1973a) is the definition of the onset of rigor, is indicated in terms of time, pH, and ATP-concentration (R-value). If the temperature of the muscle is maintained at 38°C the postmortem changes occur quite fast due to the high temperature. The loss of extensibility or onset of rigor starts at pH 6.15 and R 1.00 (about 1.9 µMol ATP/g). The shortening of the muscle starts at pH 6.3 and R 0.96 (about 2.4 µMol ATP/g muscle) proceeding slowly till the full rigor is observed. Rigor shortening begins therefore just before the onset of rigor occurs. At 25°C the loss of extensibility starts at lower pH (5.85) and ATP concentration (R 1.06 i.e. 1.2 µMol ATP/g muscle) than at 38°C. There is again a shortening starting at pH 6.1 and R 0.99 (about 2 µMol ATP/g). At 15°C the length of the loaded muscle decreases parallel to shortening; the onset of rigor as defined above occurs at pH 5.75 and R 1.12; that is at lower values of pH and ATP than at 25° and 38°C. The shortening of the muscle, however, starts right from the beginning at pH 7.0 and maximum ATP concentration. At -1°C, i.e. under extreme cold shortening conditions, the muscle shortens rapidly within 2 hours of incubation by 20 cm. Full contraction of the unloaded muscle is reached at pH 6.35 and R 1.03 (i.e. 1.5 µMol ATP/g). On loading the muscle one observes also a reduced lengthening of the muscle,



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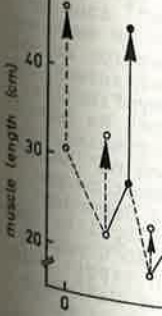

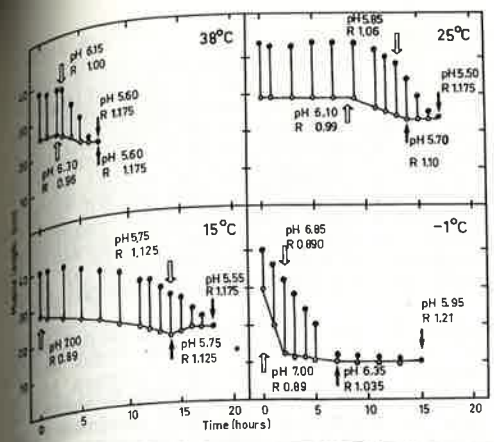

Fig. 1: Changes in extensibility and muscle length of "neck muscles" at four different temperatures of storage related to time postmortem. (o) length of unloaded muscle; (●) length of shortly loaded muscle; (▽↓) time (pH and R-value) at which loss of extensibility begins or is completed, respectively; (△↑) time (pH and R-value) at which shortening begins or is completed, respectively.

but this occurs at a slower rate than without load. After 2 hours the loaded muscle is only 10 cm less extensible whereas the unloaded muscle has shortened by 20 cm. After 7 hours of incubation the tetanus in cold shortened muscle is so strong that a load of 250 g/cm^2 cannot stretch the muscle by more than 2 cm. According to the definition of onset of rigor mortis given above at -1°C the onset of rigor seems to occur at pH 6.85 and full ATP concentration. This seems to be unreasonable. As long as a muscle can contract it should also be extensible. But the extensibility depends on the load applied (Bendall, 1973b). Using a load of 526 g/cm^2 instead of 250 g/cm^2 we found under the extreme conditions of -1°C no significant difference. As we obtained an analog but less extreme behaviour of the muscle on incubation at 5°C we carried out experiments at this temperature loading the muscles in intervalls with increasing loads.

2. The effect of load on the extensibility of cold shortened muscles

Muscles were incubated unloaded at 5°C. At different times postmortem the strips were loaded subsequently with 250 up to 764 g/cm^2 for a short period. With zero load the muscle shortened within the first hour of incubation, followed by a period where no further shortening could be observed. After 3 hours a further contracture could be recorded. This observation of the 2 phase shortening has been already described by Bendall (1973b), who reported in some cases even a relaxation of a cold shortened muscle after an initial shortening. The second phase of shortening started at pH below 6.7 ending at 13 hours at pH 6.1. The length of the muscle loaded with 250 g/cm^2 fell for the second time below pH 6.6, 0.1 pH units below the muscle with zero load. At 13 hours all extensibility was lost. With a load of 345 g/cm^2 the loaded length fell finally below pH 6.45. Under load with 764 g/cm^2 the length is reduced only below pH 6.2. This experiment shows that the cold shortening of muscles can be overcome by increasing loads. As mentioned above, the onset of rigor is defined as the beginning of the loss of extensibility of the muscle; with a load of 250 g/cm^2 the extensibility started decreasing at pH 6.3, with 345-526 g/cm^2 at pH 6.2 and with 616-764 g/cm^2 at pH 6.1. Taking these results into account we should define the moment of rigor mortis as the moment when a muscle under maximum appliable load looses extensibility. A comparison of the latter experiments with that of Fig. 1 (the experiment at -1°C) shows that at cold shortening temperatures the loss of extensibility with a load of 250 g/cm^2 apparently does not coincide with the real loss of extensibility i.e. rigor onset. If the same experimental set up as used for 5°C was applied to muscle strips at 20°C and 38°C, it could be shown that the changes observed with increasing loads at 5°C do not occur at 20° and 38°C. The determination of the onset of rigor mortis in extreme cold shortened muscle (+0°C) was elucidated with a different set up of experiments.

3. Influence of oscillating temperatures on extensibility and shortening of muscle

Fresh pre-rigor muscles were incubated unloaded for 2 hours at 0°C, stretched with a load of 250 g/cm^2 and incubated for 1 hours at 20°C without load. After loading the muscle again the temperature of incubation was lowered a second time to 0°C for 1 hour and so on (Fig. 2). Immediate chilling to 0°C induced cold shortening accompanied by a reduced length of the loaded muscles. Continuing the incubation at 20°C caused a relaxation of the unloaded and loaded muscle. This relaxation-contraction cycle proceeded in the unloaded muscle down to pH 6.3. The irreversible loss of extensibility in the muscle starts at about pH 6.1/6.0 and R 1.03. This is in agreement with the results at 5°C above, where the irreversibility of the loss of extensibility was detected by increasing the load.

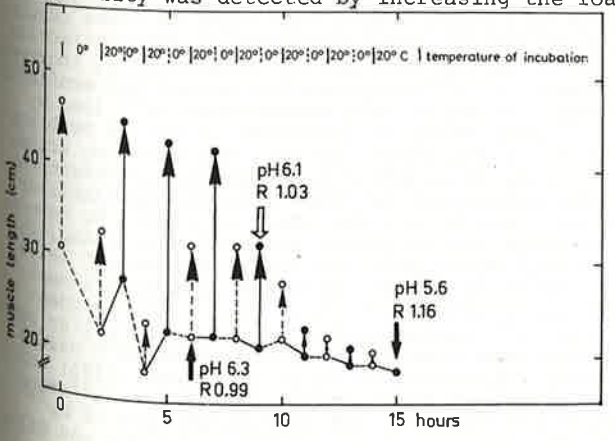

Fig. 2: Shortening and changes in extensibility of a prerigor beef muscle at temperatures oscillating between 0° and 20°C. The muscle (M.mastoideus) was incubated at 0° and 20°C as described in the text. It was loaded with 250 g/cm^2 only. Arrows mean the same as in Fig. 1.

4. The influence of temperature on the onset and completion of rigor

The results presented in Fig. 1 for 4 temperatures already indicate that the beginning and total loss of extensibility of a muscle depends on its temperature. In Fig. 3 the influence of all measured temperatures between -1^{o} and 38^{o}C on the onset and full development of rigor is shown with regard to pH and R-value (ATP concentration). With regard to pH (Fig. 3A), the onset of rigor at 38^{o}C starts at pH 6.25 reaches a minimum at 12-15^{o}C with pH 5.75 and increases again to pH 6.1-6.2 at 0^{o}C. The total loss of extensibility (completion of rigor) is reached between 8^{o} and 38^{o}C at pH 5.5-5.6 increasing to pH 5.9-6.0 at 0^{o}C. With regard to R-value (Fig. 3B) the onset of rigor at 38^{o}C starts at about R 0.95 (2.9 µMol ATP/g muscle) reaching a minimum at about 15^{o}C at R 1.08-1.10 (about 0.9 µMol ATP/g). At 0^{o}C again the irreversible loss of extensibility starts at R = 1.0 as at 38^{o}C. The development of rigor is completed at R=1.17 (less than 0.5 µMol ATP/g) at all incubation temperatures.

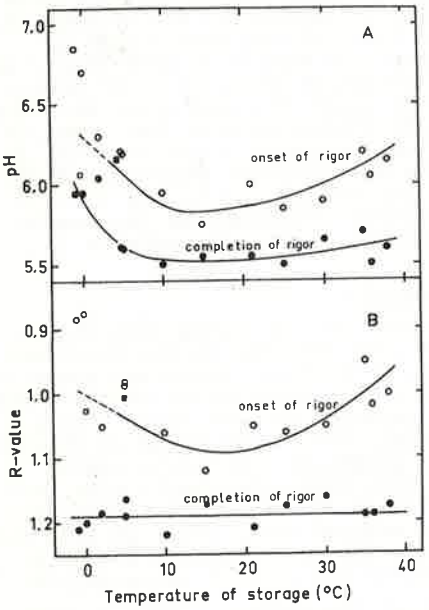

Fig. 3: pH (A) and R-value (B) at which irreversible loss of extensibility begins (o) or is completed (●) in "neck muscles" stored postmortem at various temperatures (see arrows in Fig. 1). (□) beginning of loss of extensibility of muscles stored at 0^{o}C and periodically (1-2 h) warmed up to 20^{o}C derived from Fig. 2. (■) value at 5^{o}C derived from experiments described in chapter 2.

It is noticeable that rigor completion occurs at the same ATP concentration for any given temperature but not at the same low pH. The reason for this fact is a different R-value/pH relationship during the time postmortem at temperatures below 5^{o}C compared with higher temperatures.

5. Influence of temperature on the shortening of muscle

The shortening of unloaded muscles incubated at various temperatures shows a different behaviour than the loss of extensibility. All results obtained are presented in Fig. 4. At temperatures above 15^{o}C shortening does not start at pH values above 6.25. Below 15^{o}C the muscle starts shortening right after incubation at pH 7.0. Above 15^{o}C 50 % shortening is reached at pH 5.9-6.0, full shortening at pH 5.6-5.7. Below 15^{o}C 50 % shortening is obtained with falling temperature of incubation at increasing pH-values reaching pH 6.9 at -1^{o}C. The full shortening occurs at rising pH-values in a parallel fashion to the 50 % shortening value; it is reached at pH 6.35 for -1^{o}C and at pH 5.6 for 38^{o}C.

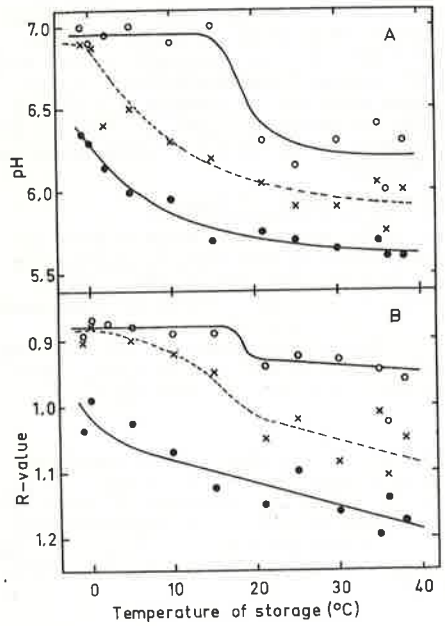

Fig. 4: pH (A) and R-value (B) at which shortening begins (o), is completed (●) or reaches 50 % of maximum final shortening (x) in "neck muscles" stored postmortem at various temperatures (see arrows in Fig. 1).

With regard to R-values the shortening starts at fairly low values (0.89-0.96 equivalent to about 2.5-3.0 µMol ATP/g muscle) over the whole range of temperatures studied (Fig.4). Above 15^{o}C R-values for the 50 % shortening and full shortening run nearly parallel to those for the beginning of shortening; below 15^{o}C in the temperature range of cold shortening the 50 % shortening value is reached at high ATP concentrations, below 5^{o}C the full shortening is reached already at about 2 µMol ATP/g muscle (i.e. R = 1.0).

Discussion

Between 38^{o} and -1^{o}C all unloaded prerigor muscles show a shortening (Fig. 4) but to a different extent (Fig. 1). Also time postmortem, pH and ATP concentration of the beginning of shortening are different. In general shortening of muscles should take place before the onset of rigor occurs as it needs a sufficient ATP concentration for contraction and separation of myosin from actin. Additionally, shortening needs an increase in Ca^{2+}concentration around the myofibrils. The sarcoplasmic reticulum (SR) accumulates Ca^{2+} ions by means of a Ca^{2+} pump which is localized in the SR membrane and is driven by an ATP/ATPase system. The question arises why in the presence of an ATP concentration sufficient for contraction the release of Ca^{2+}ions into the myofibrillar space is taking place before the onset of rigor? We have to distinguish between two different kinds of shortening, the "rigor shortening" above 20^{o}C and "cold shortening" below 15^{o}C. Rigor shortening can be explained by a most recent report of Whiting (1980) which shows that a pH dependent increase of the Ca^{2+}-concentration in the myofibrillar space during the postmortem changes in muscle above 20^{o}C causes rigor shortening. According to Buege and Marsh (1975) cold shortening is explained by an anoxic release of Ca^{2+}-ions from the muscle mitochondria and an reduced rate of Ca^{2+} uptake by the SR. Pearson et al. (1973) and Davey and Gilbert

(1974) suggested that cold shortening is primarely due to a leakage of Ca^{2+} from the SR. Cornforth et al. (1980) now could show that apparently both explanations are correct because both processes interfere with each other leading to cold contracture of prerigor muscle. The Ca^{2+}-ions, however, are released for a different reason. Cold contracture can be induced at any time postmortem in prerigor muscle, rigor shortening is limited to the late prerigor phase. Shortening is due to the release of Ca^{2+}-ions in the presence of a sufficient ATP concentration causing a build-up of tension within a muscle. At temperatures above 20°C the degree of shortening (Locker and Hagyard, 1963) and consequently the build up of tension is small, whereas in the cold shortening range below +15°C the tension and shortening increases with falling temperature. The high tension leads in cold shortened muscles under medium load to an early loss of extensibility, which can be overcome by increasing loads or rising the temperature. Both, increasing loads and temperature show that the loss of extensibility in cold shortened muscle is not identical with the onset of rigor. From our results it becomes obvious that the irreversible loss of extensibility or the onset of rigor takes place after the ATP concentration and pH fall below certain values which change with temperature.

Conclusions for the handling of meat

Shortening in a muscle can be kept at a minimum if 20°C are reached as fast as possible. This is important for the handling of hot boned meat. Also the drip loss is at a minimum if temperatures of 10-15°C are reached as soon as possible (Honikel et al., 1980). The onset of rigor is the critical point for the salting of "hot" beef (Honikel et al., 1981; Hamm, 1981). Meat at 35°C must be salted above pH 6.25 which is reached within 3-4 hours postmortem. If the meat can be cooled rapidly to 15°C, then the rigor sets in at pH 5.75 which occurs 10-15 hours postmortem.

References

Bendall, J.R. (1973a). In: The Structure and Function of Muscle (ed. G.H.Bourne). Academic Press, New York and London, 2nd edition, p. 241.

Bendall, J.R. (1973b). Proc. 19th Europ.Meat Res.Workers Meeting, Paris, p. 1.

Buege, D.R. and Marsh, B.B. (1975). Biochem. Biophys. Res.Commun. 65, 478.

Cornforth, D.P., Pearson,A.M. and Merkel,R.A. (1980). Meat Sci. 4, 103.

Currie, R.W. and Wolfe,F.H. (1979). Canad. J. Animal Sci. 59, 639.

Currie, R.W. and Wolfe,F.H. (1980). Meat Sci. 4, 123.

Hamm, R. (1981). In: Developments in Meat Science - 2 (ed.R.Lawrie). Appl. Science Publ. Ltd. Barking, U.K., p. 93.

Honikel, K.O. and Fischer, C. (1977). J. Food Sci. 42, 1633.

Honikel, K.O.,Fischer, C. and Hamm, R. (1980). Fleischwirtschaft 60, 1577.

Honikel, K.O., Fischer, C., Hamid, A. and Hamm, R. (1981). J. Food Sci. 46, 1.

Jolley, P.P., Honikel,K.O. and Hamm, R. (1980/81). Meat Sci. 5, 99.

Locker, R.H. (1960). Food Research 25, 304.

Locker, R.H. and Hagyard, C.J. (1963). J. Sci. Fd. Agric. 14, 787.

Locker, R.H. and Daines, G.J. (1975). J. Sci. Fd. Agric. 26, 1721.

Marsh, B.B. and Leet, N.G. (1966). J. Food Sci. 31, 450.

Pearson,A.M., Carse, W.A., Davey,C.L., Locker, R.H, Hagyard,C.J. and Kirton, A.H. (1973). J. Food Sci. 38, 1124.

Powell, V.H. (1978). Proc. 24th Europ. Meat Res. Workers Meeting. Vol.I, paper D 1.

Whiting, R.C. (1980). J. Food Sci. 45, 288.

Davey, G.L. and Gilbert, K.V. (1974). J. Fd. Technol. 9, 51.