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INFLUENCE OF TEMPERATURE ON THE RATE OF POST-MORTEM METABOLISM AND WATER-HOLDING CAPACITY OF BOVINE NECK MUSCLES

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SUMMARY

Strips of forty bovine neck muscles were placed at temperatures in the range −1° to +30°C within 45 min of slaughter and stored for up to 24 h. Strips were taken at various times during storage and assayed for pH, 'R' value (degree of transformation of ATP to IMP) and ATP concentration. The water-holding capacity (WHC) of the intact muscle was compared with the WHC of a salted muscle homogenate prepared at each sampling time. The rate of pH fall post mortem was relatively low around +5°C and increased at lower or higher temperatures. ATP concentration showed a delay phase dependent on storage temperature and a subsequent rate of depletion which was also temperature dependent. The patterns of change in WHC of the muscle samples and the salted homogenates differed, the former showing a rapid fall to a fairly steady level shortly after initiation of storage, the latter showing no appreciable change until the onset of rigor. It is suggested that salting meat at any time prior to the onset of rigor will confer improved WHC and that the temperature of storage post mortem should be chosen to induce low rates of ATP turnover so as to prolong the feasible delay between slaughter and salting.

INTRODUCTION

Beef in the pre-rigor state can be processed to produce sausages of excellent quality (Hamm, 1972). This effect, which is often referred to as the 'pre-salting effect', has been shown to originate from the combined influence of comparatively high levels of ATP (adenosine triphosphate) and high pH present in normal muscle tissue shortly after slaughter (Hamm, 1956). In a relaxed muscle, ATP is responsible for keeping the actin and myosin filaments apart and thus providing a high degree of

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immobilisation of water in the tissue (Hamm, 1972). This high WHC (water-holding capacity) may be retained for several days by salting the minced pre-rigor meat (Hamm, 1957, 1958) despite the fact that both ATP breakdown and glycolysis are accelerated by both mincing and salting (Hamm & van Hoof, 1971; van Hoof & Hamm, 1973; see also review by Hamm, 1977; Honikel & Hamm, 1978). Thus, it appears that the concentration of ATP in the muscle tissue at the time of processing or pre-salting is of paramount importance for the WHC of meat and the quality of products made from fresh or pre-salted material. Hamm (1972, 1977) postulated that the pre-salting effect is due to an inhibition of rigor development in fibre fragments and filamental material under the combined influence of ATP, high pH and high ionic strength in the pre-rigor salted tissue. It is unlikely to result from appreciable amounts of dissolved myofibrillar proteins (Hamm & Grabowska, 1979).

Most of the investigations on the WHC of pre-salted muscle have naturally concentrated on muscle processed as soon as possible after slaughter. Similarly, post mortem glycolysis has been studied extensively in either intact muscle (e.g. Bendall, 1973*b*) or ground muscle, with or without the addition of salt (Hamm, 1977). As far as we are aware, no attempt has been made to relate the post-mortem fall in ATP and pH in the intact muscle to changes in WHC in intact muscle and a sausage-type homogenate made from this muscle under various regimes of time and temperature. Such information is of practical importance in determining the length of time that hot deboned beef may be left at a particular temperature before salting and retain a marked benefit over conventionally chilled, post-rigor material. In this paper we present results of studies in ATP breakdown and glycolysis in beef neck muscles stored at different temperatures post mortem and discuss their relationship to the WHC of unheated muscle and the WHC of unheated salted muscle homogenates. The relationship between the rate of post-mortem metabolism and the WHC of heated muscle systems will be presented in a later paper.

MATERIAL AND METHODS

Material

Beef neck muscles (mainly the *M. sternomandibularis* and *M. sternomastoideus*) of about forty animals were obtained from the local abattoir within 40 min of slaughter and trimmed of fat and connective tissue. The muscular tissue was then divided longitudinally into samples of approximately 90–200 g in slices of 1–2 cm thick. One of these strips was analysed as detailed below, to give the zero time values, the remainder being sealed in individual polypropylene pouches and placed in a cryostat bath at the desired temperature. At selected times during storage and at 24 hours post mortem, one pack, selected at random, was removed for sampling. Results at each temperature were obtained with muscles from a different animal.

Determination of pH

pH was determined after homogenising 3–5 g of muscle with an approximately equal amount of double distilled water. The pH measurements were made immediately after homogenisation with a Präzisions pH-Meter 391 (Wiss. Tech. Werkstätten, Weilheim, Germany) and a combined glass electrode.

Determination of breakdown of ATP

Perchloric acid extracts were produced by the method of Dalrymple & Hamm (1973) and frozen in a domestic deep freeze until analysed. ATP was determined in this extract enzymically (Jaworek *et al.*, 1970).

An indication of the degree of accumulation of inosine monophosphate was obtained by diluting 0·1 ml of the extract with 1·9 ml 0·1M phosphate buffer, pH 7, and determining the ratio of absorption at 250 and 260 nm. This ratio is referred to as the 'R' value (Honikel & Fischer, 1977). For each experiment, graphs were made of (a) ATP versus time, (b) pH versus time, and (c) 'R' value versus time. Slopes of linear portions of graphs (a) and (b) were determined by linear regression analysis.

Water-holding capacity of unminced muscle

Water-holding capacity was determined on 0·3 g of the intact muscle using the filter-paper press method (Grau & Hamm, 1952).

Water-holding capacity of salted muscle homogenates

Following removal of sufficient material for determination of pH, metabolites and the WHC of the intact muscle as detailed above, the remaining muscle was minced once through a plate with 4·5 mm holes. The mince was weighed and mixed by hand with 3% by weight sodium chloride. Iced water, 50% by weight of the original mince, was added to the salted mince, lightly mixed by hand and homogenised in a 'Moulinette' (Moulinex, France) cutter. The resultant salted muscle homogenate therefore had a final salt concentration of 2%. The WHC of this homogenate was assessed in a similar manner as the unminced muscle. The term 'homogenate' is used for convenience and is not meant to imply a perfectly homogeneous entity histologically or chemically.

RESULTS AND DISCUSSION

Changes in pH

The mean pH at the beginning of storage was 6·84 with a standard deviation of 0·12. Fall in pH was generally linear until pH 5·9–5·7, as reported by Bendall (1973*a*,*b*), the mean pH after 24 hours' storage at all temperatures being 5·64 (standard deviation = 0·18). Bendall (1973*b*) also reported a slower rate of fall in pH during the early stages of post-mortem metabolism when ATP is being resynthesised

from creatine phosphate, a noticeable increase in rate of pH fall occurring when resynthesis through this mechanism fails. Such a two-stage pH/time relationship was discernible in the present study in most (but not all) of the experiments conducted in the range 5–20 °C. It was not generally discernible outside this range, which was expected in view of the known acceleration of post-mortem muscle metabolism arising from increase in temperature on the one hand and cold-shortening on the other. This pattern is not always reported in any event (e.g. Jeacocke, 1977; Honikel & Hamm, 1978).

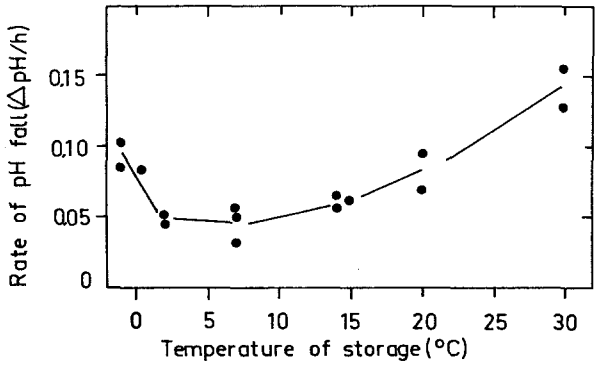

Fig. 1. Calculated rates of pH fall between pH 6·8 and 6·1 in beef neck muscle held at various temperatures. Only those slopes with $p < 0{\cdot}05$ or better are shown.

The rate of change in pH obtained by linear regression analysis for fifteen muscles with inital pHs of 6·8 to 6·9 is shown in Fig. 1. The shape of the curve and the values obtained are in good agreement with those of Jeacocke (1977).

Changes in ATP concentration and 'R' value

The mean initial ATP concentration (2·52 μmol/g) was far lower than generally reported (e.g. Bendall, 1973*b*; Tarrant & Mothersill, 1977) and an explanation of this discrepancy cannot be provided. The accepted two-stage pattern of ATP depletion (Bendall, 1973*a*), consisting of an initial delay phase (during which ATP depletion is matched by resynthesis), followed by a linear fall in concentration, was clearly discernible in the majority of the experiments; an example of this is shown in Fig. 2. Thus, the ATP concentration at any time post slaughter was the result of two factors: (a) the length of time during which the delay phase was operative and (b) the subsequent rate of ATP depletion. The length of the delay phase was dependent on the temperature of storage, as shown in Fig. 3, although variation between samples from different animals stored at the same temperature was marked in this parameter, particularly at the extremes of the temperature range studied.

At −1 °C there was little or no delay phase, in the range 5 °–18 °C it lasted for 6–8 h after initiation of storage, returning to low values at 30 °C. At the end of the delay

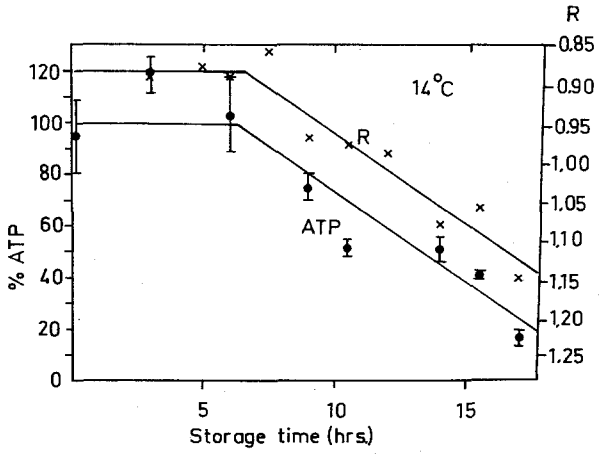

Fig. 2. Example of changes in concentration of ATP as a percentage of the delay phase during storage at 14 °C. Extractions were in duplicate, error bars indicating range. The corresponding changes in 'R' value are also shown. (100% ATP = 2·8 μmol ATP per gramme). ●, ATP; ×, 'R' value.

phase, the subsequent rate of ATP depletion was fairly independent of storage temperature over the range +0·5 °C–20 °C, and at a low level (Fig. 4). Outside this range the rate increased considerably.

In each experiment indicated in Fig. 4 the value for 100% ATP was derived by studying the relevant graphs of ATP concentration against time and taking the mean for all points considered to be part of the delay phase. Such a procedure is useful in comparing animals but inevitably introduces errors. Assessing the state of ATP degradation to IMP in a particular sample by determining the 'R' value greatly reduces the effect of these sources of error. The overall correlation between per cent

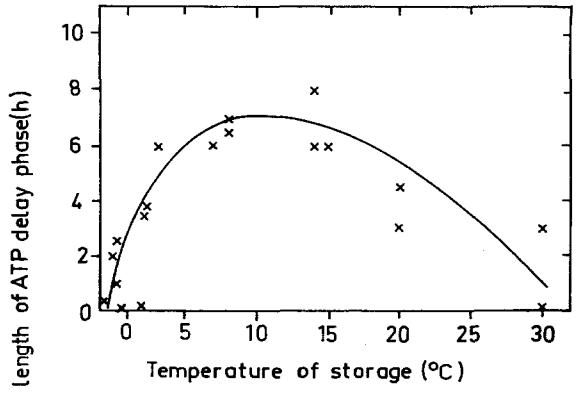

Fig. 3. The time of delay phase where the ATP concentration remains constant at its initial level in beef neck muscles held at various temperatures.

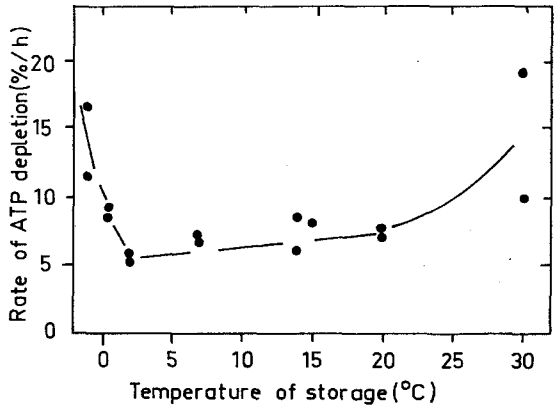

Fig. 4. The calculated rates of ATP depletion in beef neck muscle held at various temperatures. Only those slopes with $p < 0{\cdot}05$ or better are shown. The ATP concentration in the delay phase was taken as 100%.

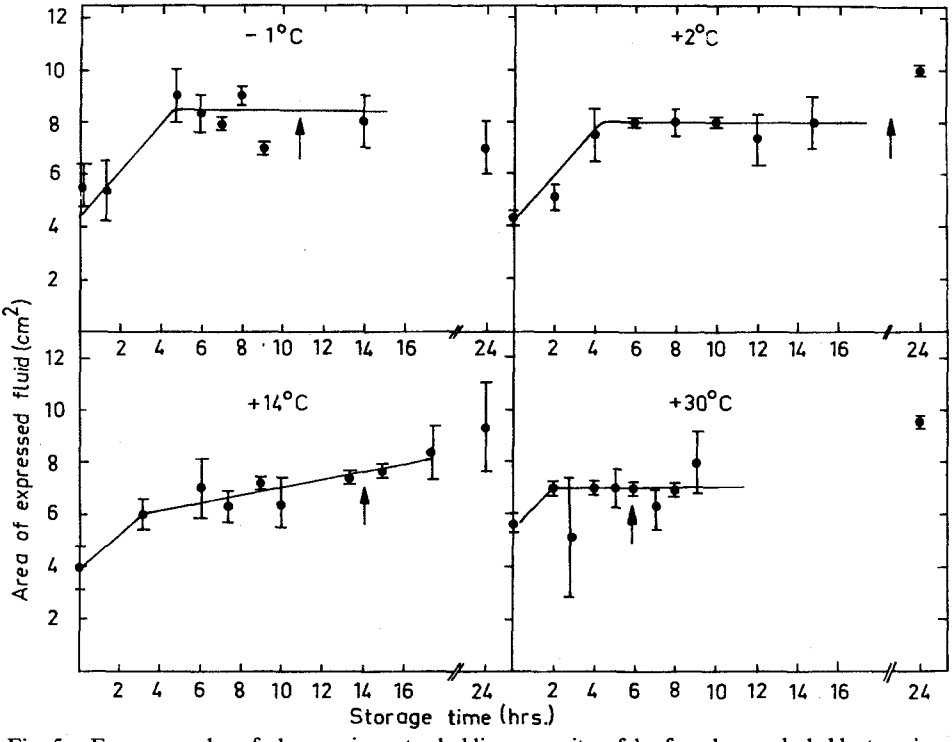

Fig. 5. Four examples of changes in water-holding capacity of beef neck muscle held at various temperatures. (−1 °C; 2 °C; 14 °C; 30 °C). Error bars indicate range of duplicate determinations. Time at which a pH of 5·9 was reached in the muscle samples is indicated by arrows.

ATP and 'R' value was high ($r = -0{\cdot}869, p < 0{\cdot}001$) and examination of the curves in Fig. 2 shows that the use of this factor provides comparable information with that achieved by analysis of ATP concentration; its use can therefore be recommended where comparatively large numbers of muscle samples are to be used in the pre-rigor state, provided that the actual values of ATP concentration are not required. In agreement with these results, the curve which Honikel & Hamm (1978) obtained by plotting 'R' value measured in the intact muscular tissue after 3·5 hours post mortem versus storage temperature is quite similar to that in Fig. 4.

Changes in water-holding capacity of muscle and water-holding capacity of salted muscle homogenates

WHC in the muscular tissue of the majority of the experiments fell during the first few hours of storage and then remained virtually constant (Fig. 5). The arrows on Fig. 5 indicate the approximate time at which pH 5·9 was observed. At this pH at least 40% (about 1 μmol ATP per gramme) of the intial ATP was still present and therefore serves as an indicator of the onset of rigor (Bendall, 1973*a*). It can be seen that onset of rigor had apparently no additional effect on WHC. Figure 5 also demonstrates that temperature of storage appeared to have no marked effect on the overall pattern of change, nor did the comparatively high level of ATP concentration during the delay phase (by comparison with Fig. 3). Initial pH and ATP concentration did not affect WHC and there was no obvious effect of temperature of storage on the WHC at 24 h.

The pattern of change of WHC post mortem in salted muscle homogenates was entirely different from that of the intact muscles from which they were prepared (Fig. 6), in that there was no free water expressed from samples of homogenates prepared after several hours' storage and therefore no change in WHC could be observed. The arrows again indicate the approximate time at which pH 5·9 was observed in the intact muscle and it is obvious that marked changes in the salted muscle homogenates do not occur much before the onset of rigor mortis. (Note that, because the examples in Fig. 6 do not necessarily correspond to those in Fig. 5, the indicated time at which pH 5·9 was observed differs slightly between the two figures.)

In that the results for ATP, 'R' value and pH generally agree with other published findings, the muscle samples may be regarded as typical. Nevertheless, the results on WHC appear to disagree with the earlier work of Hamm (1956), in which he recommended that meat should be salted as rapidly as possible post slaughter (i.e. while the ATP was still high) in order to retain the maximum benefit of the pre-salting effect, as he found that ATP was mainly responsible for the good WHC of pre-rigor meat. The time scale of change in WHC of the muscle sample (Fig. 5), with a rapid drop early in storage, is certainly consistent with Hamm's (1956) findings, but it is difficult to reconcile the lack of effect of storage temperature on WHC with the varying ATP delay phases (Fig. 3).

The WHC of the salted muscle homogenates seemed to be governed by the

development of rigor mortis. Hamm (1972, 1977) has suggested that the high WHC of pre-salted meat might be due to a strong electrostatic repulsion between the dissociated myofibrillar proteins, myosin and actin, caused by the combined influence of ATP, high pH and increased ionic strength resulting from the salt added. It is conceivable that as long as there is sufficient ATP present in the muscle to prevent appreciable cross-linking between actin and myosin (i.e. pre-rigor),

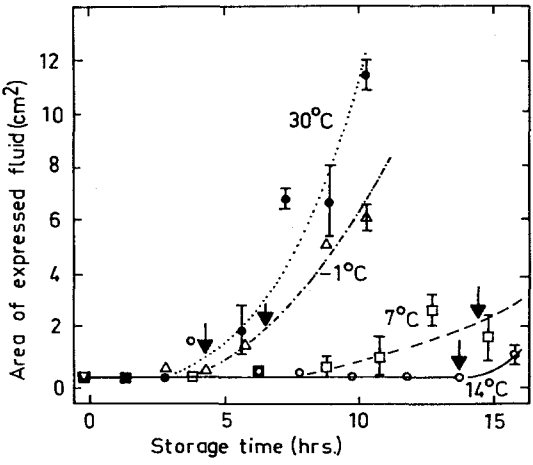

Fig. 6. Four examples of changes in water-holding capacity of beef neck muscles held at various temperatures and then homogenised with 2% salt and 50% added water. Error bars indicate range of duplicate determinations. Time at which a pH of 5·9 was reached in the muscle samples is indicated by arrows. (—·—△—·— 1 °C; — — □ — +7 °C; —○— 14 °C; ··· ● ··· 30 °C).

sufficient repulsion resulting from the increase in ionic strength by salting may be sufficient to produce the superior WHC. This crucial role of the high ionic strength would allow for different patterns of change in WHC post mortem measured with or without the addition of salt, as reported here, and in this context it is worth noting that Hamm's findings (1956) were based on unsalted material. The effect of declining pH, pre-rigor, may only be noticeable if more water is added to the homogenate to reduce the ionic strength (Grau & Hamm, 1954). Such an effect is indeed noticed, using the filter-paper press method, if the amount of salt added with the same volume of water is reduced (Jolley *et al.*, in preparation).

It appears that a markedly better WHC can be achieved over post-rigor meat by salting at any time prior to the onset of rigor. The postulated effect of declining pH means that best results will be achieved by salting as soon as possible after slaughter, but this is a secondary consideration. Storage at temperatures that correspond to the slowest rates of ATP turnover is clearly indicated, concomitant with microbiological considerations.

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