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PREDICTING GLYCOLYSIS DURING A FREEZE-THAW CYCLE IN PRE- AND POST-RIGOR BEEF MUSCLES

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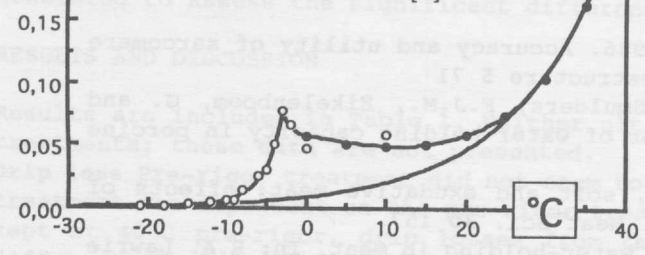
ABSTRACT: Freezing meat pre-rigor and subsequent thawing reduced its pH. Modelling the decline in pH with respect to the temperature profile showed that the majority of the decline occurred during the latent phases at about 5°C during freezing and at -2°C during thawing. Small muscle blocks at pH 7.0, when frozen at -70° and then thawed at 4°C, had a pH after thawing of about 6.0. For measurements which are affected by rigor, freezing before full rigor should be avoided. When essential, freezing meat at a lower pH was shown to have less effect on the final pH after thawing. Slower thawing was predicted to cause a greater reduction in pH than faster thawing. Careful consideration should be given to any conditions of freezing and thawing and the effects they may have on the measurements to be made.

BACKGROUND: Post-rigor freezing is often used conveniently when large numbers of samples or numerous assessments have to be made. The effects on structure, proteins and quality are generally small and can be minimised by fast freezing, by minimising temperature fluctuations during storage and by rapid thawing. However, freezing pre-rigor muscle is known to affect the rate of glycolysis and enzyme activities, and thawing such meat will produce a larger amount of drip and produce tougher meat than chilling. Several mechanisms, such as structural damage due to ice crystal formation and changes in metabolism due to freeze concentration of enzymes and substrates, may operate and change rapidly the composition of the muscle. It is important therefore to choose a freeze/thaw cycle which minimises these changes.

OBJECTIVES: Use modelling to predict the pH after a freeze/thaw cycle applied to pre- and post-rigor meat. Show the kinetics of the decline in pH in the different stages during freezing and thawing. Design suitable methods or equations for predicting those methods of freezing prior to experimentation.

METHODS: *M. Sternomandibularis, longissimus dorsi* (Ld) and *diaphragma* were excised within 1 hour after stunning and stored at 22°C. At various times, 25 or 50g blocks (20 mm wide by 50 mm long) were cut, a thermocouple placed at the centre, placed in a plastic pouch and frozen in still air at -70°C. When the temperature at the centre of the block was below -40°C, the meat was thawed in still air at 4°C. When just completely thawed, 1g of meat was homogenised for 20 sec in 9ml 150mM NaCl and the pH measured within 30 sec. To predict the changes in pH during the freeze/thaw cycle, the rates of pH decline in beef were plotted in relation to the temperature and empirical equations derived relating the rate of pH decline to temperature. Equations for temperature changes were derived similarly from temperatures measured in the centre of the blocks throughout the freezing and thawing cycle.

RESULTS: Figure 1 shows the rate of pH decline as a function of temperature. The upper curve was derived from literature [1, 2] for temperatures above 0° and for temperatures below 10°C [3]. The curves were appended and standardised to the same rate at their average values at 5°C. This curve shows that the rate of pH decline decreases from 30° to a minimum at about 10° below which the rate rises up to a peak at about -3°C. The rate of decline in pH at -3°C is equal to that at about 25°C. At lower temperatures, the rate decreases exponentially with decrease in temperature with a very low rate below -30°C. A further exponential curve (lower curve, Figure 1) was constructed by combining only the values above 25°C with those below -10°C.



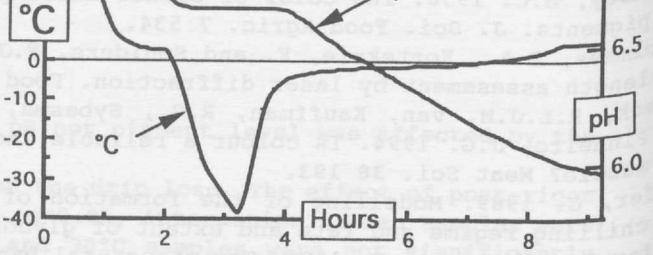
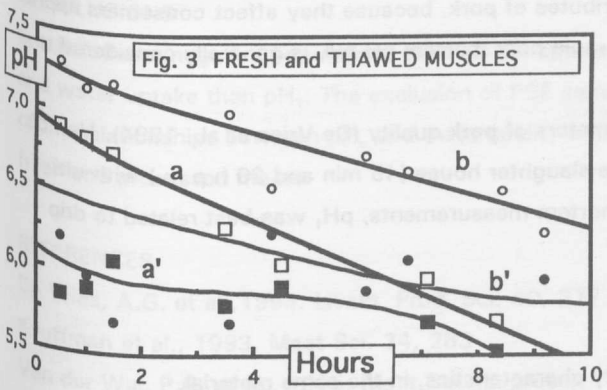
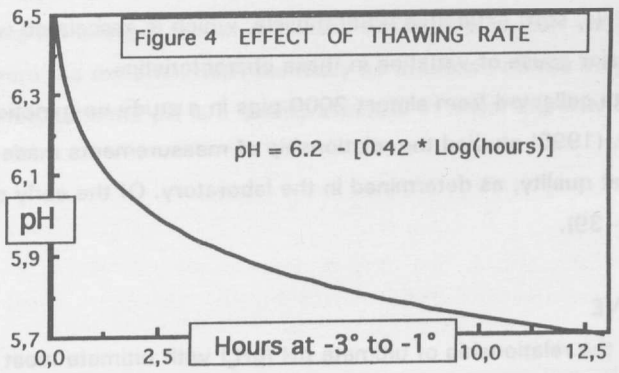


Figure 2 shows the cooling of 25 g of pre-rigor meat, initially, at 22°, when stored at -70°C. The temperature decreased quickly to a plateau at about 5°, remaining there for almost 1 hour. Later the temperature declined sharply to -40° in a further 1 hour. When thawed, the temperature rose quickly to about -5° in 1 hour and then passed through the freezing point (about -2°C) and reached 0°C after a further 4 hours.

When determined experimentally, the pH of Ld samples (open squares, Figure 3) decreased approximately in proportion to the time of storage at 22°C from about pH 7.0 at 30 min to 6.0 at 7 hours, that is, at a constant rate. The rate, however was about 2.2 times faster than that of the literature values (Figure 1). The curves in Figure 1 were then rescaled proportionately to give the observed rate at 22°C (curve a, Figure 3). After freezing and thawing (filled squares), the observed pH decreased to about 6.0 when frozen early post mortem and to about 5.6 when frozen 8 hours after slaughter at pH 5.7. For 25 g of *Diaphragma*, frozen at pH 6.7, the pH was reduced to 6.3 by a similar freeze/thaw cycle. The pH of Ld, when frozen and thawed to 4°C, was calculated by integrating the temperature (Figure 2) with the rates (Figure 1). The upper curve (Figure 1) was used when the pH was above 6.2 and the lower curve for pH values of 5.9 or below. Between these pH's, the rate was changed from the upper curve to the lower, in proportion to the pH between these limits. Using this, the calculated pH values after thawing (curve a', Figure 3) agreed well with the observed values. For *Sternomandibularis* (50 g), the pH declined more slowly than that of the Ld, from 7.3 initially to 6.5 at 9 hours (open circles, Figure 3). The predicted effect of freezing and thawing on the pH was then calculated (curve b', Figure 3) in the same way as for the Ld and showed a good agreement with the observed pH at later times but was higher, by about 0.4 unit, than the observed pH (filled circles, Figure 3) when frozen at the early times post-mortem.





The curves in Figure 1 were used similarly to calculate the pH decline during a freeze/thaw cycle (Figure 3). The decline in pH, from 7.0 before freezing, was about 1.0 unit throughout freezing and thawing cycle. A decrease of about 0.3 unit was calculated to occur during freezing, mostly during the time at 5°, and then a further decline of 0.7 unit was predicted during the thawing, mainly at the freezing temperature (Figure 2).
Similarly, the effect of increasing the time at the freezing point was calculated (Figure 4) and showed that, starting at pH 7.0 and with the same freezing times, the decline in pH was approximately related to the log of the time at the freezing point (-4° to -1°). The final pH after freezing and thawing, decreasing to 6.4 with 0.7 hour at the freezing point and decreasing to 5.8 with about 10 hours at the freezing point.

DISCUSSION: The two curves used for calculation of the effect of a freeze/thaw cycle were chosen to represent pre-rigor and post-rigor conditions. Freezing of pre-rigor meat give a rate at -3°C about equal to that at 25°C. No direct comparisons of these temperatures appear to have been made but the rate at -3° is known to be higher than that at 15°C [3]. The decrease in pH at -3° is equivalent to that at about ambient and occurs in a variety of other species [3]. The mechanism of this high rate is uncertain, and may originate from the impared functionality of cell membranes by ice crystal formation, but was modelled as an extension of the curves at low chill temperatures which give increased rates and muscle contraction. This mechanism would not operate when the muscle was unable to contract, that is, at about pH 5.9 or below which was represented by the second curve. This satisfied the observed lower reduction in pH by freezing and thawing meat at a lower initial pH. Large differences in rates of pH decline and in the effect of freeze/thaw cycle of the thawed pH were observed between the muscles. Some of the differences were due to the different temperature profiles dependent on the ability of the sample to rest at the freezing temperature. In practice wider variations in final pH would be expected due to variations in cooling at different positions within the freezer and chiller and variations in packing. Measurements of factors affected by rigor will then be more variable and changed with freezing and thawing.
Freezing and thawing pre-rigor muscle may therefore have significant consequences on the composition because changes in enzyme activities are induced by freezing [5]. For the calpain enzyme system, calpain and calpastatin decrease during rigor development and a faster decrease occurs with faster rigor development. Freezing and thawing pre-rigor meat, therefore, may reduce the level of calpain and calpastatin by autolysis [4] or by other degradative processes [6].

REFERENCES:

1. Marsh, B. B. 1954. *J. Science Food Agriculture,* **5**, 127.
2. Bate-Smith, E. C. and Bendall, J. R. 1956. *British Medical Bulletin,* **12**, 230.
3. Behnke, R. J, Fennema, O. and Cassens, R. G. 1973. *J. Agricultural Food Chemistry*, **21**, 5.
4. Dransfield, E. 1994. *Meat Science,* **37**, 391.
5. Rhodes, D. N. 1961. *J. Science Food Agriculture,* **12**, 224.
6. Parkes, C., Kembhavi, A. A. and Barrett, A. J. 1985. *Biochemical J.*, **230**, 509.