



## Very Fast Chilling of Beef and Tenderness—a Report From an EU Concerted Action

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### ABSTRACT

*Very Fast Chilling (VFC) in Beef is a European Union funded concerted action begun in November 1994. Scientists from the Union have met several times to review their work. Muscle is defined as VFC when it is chilled to  $-1^{\circ}\text{C}$  by 5h post mortem. The sub-zero conditions necessary will produce temperature gradients in all muscles and these give rise to considerable variation in biochemical and physical parameters. Calcium ions released by the cold within muscle fibres stimulate proteolytic action which tenderises the meat, but also stimulate contraction which toughens meat. The studies considered here suggest that a combination of proteolysis and crust freezing can produce tender beef. More research is needed into the biochemical mechanisms and pilot scale investigations could be undertaken. The early cutting and processing of the beef side would improve abattoir efficiency but VFC technique is not yet ready for full-scale industrial implementation. Copyright © 1996 Published by Elsevier Science Ltd*

### INTRODUCTION

Since December 1994 the European Union has funded a concerted action to coordinate investigations on Very Fast Chilling, VFC, in beef. Fast throughput is important for profitability in the beef industry, but fast chilling has been shown to produce considerable toughening and severe consumer reaction since tenderness is the consumer's primary concern (Ouali, 1990). Bendall (1974) advised slow cooling beef, and lamb, post-mortem so that the meat temperature did not fall below  $10^{\circ}\text{C}$  sooner than 10 hours post mortem to prevent toughening. This rule-of-thumb for slow cooling has been widely taken up in industry in the last twenty years in an effort to guarantee tenderness (Lochner *et al.*, 1980).

Today, in Ireland and in Britain and further afield, factories are given detailed specifications by the buyers from the supermarket companies. Beef sides must be cooled slowly and then suspended after dressing from the pelvic girdle to stretch the major steak muscles around the hip as they pass into rigor. Thereafter the sides are aged for 14 days, then boned and the prime cuts are aged in vacuum pack for a further 14 days or longer to achieve full maturation. Electrical stimulation may also be required, to exhaust the reserves of glycogen in the muscle that would otherwise be available to power 'cold-shortening' (Takahashi *et al.*, 1984).

These multiple component specifications produce tender beef steaks and roasts but they are expensive. Slow chilling introduces extra microbial hazard as well as increasing

evaporative weight loss. Electrical stimulation may not always be effectively applied, for example through inadequate contact at the nasal septum or at the bung, or in low voltage post dehiding, imprecise stimulation of particular nervous pathways. Moreover its effects may be influenced by other factors such as variable glycogen levels arising from varying pre-mortem stress and innate metabolic differences between different muscles such as their ratios of red to white fibres (Hertzman *et al.*, 1993). A simpler fast chill system which did not toughen meat could improve efficiency and facilitate price competition.

'Cold toughening' is the term recommended (Taylor, 1987) for use when meat is toughened by cold; 'cold shortening' should only be used when there is direct evidence of shortening. The causal mechanism, in microstructure, of cold-toughening may include increased overlap, shorter sarcomeres, and thus opportunity for increased actin-myosin lateral cross linking of contractile filaments in *rigor mortis*. This would confer greater internal cohesion and thus toughness. Shortening may occlude certain parts of the structure from attack by proteases and the lower temperature would reduce the rate of attack. Lower temperature may affect the rate of glycolytic breakdown of stored carbohydrate and thus the rate of pH fall. pH will affect enzymic rates (Goll *et al.*, 1995).

Davey & Garnett (1980) showed that cold toughening in lamb, (though they write 'cold-shortening'), could be prevented by freezing in less than 4 hr *post-mortem*. Frozen storage at  $-12^{\circ}$  for more than 10 days would prevent subsequent thaw-shortening and toughening. The mechanism that they suggested was that surface hardening produced by cold imposed a restraint on shortening.

Bowling *et al.* (1987) subjected beef sides to rapid chilling under air at  $-70^{\circ}$  for 5h, followed by equilibration at  $16^{\circ}$  for 4h. Control sides were conventionally chilled, under air at  $-7^{\circ}$  for 24h. All meat was aged for 10 days at  $2^{\circ}$ . Loin steaks (*m. longissimus dorsi*) that attained  $-2^{\circ}$  to  $0^{\circ}\text{C}$  in about 5h, from rapid chilled sides had slightly longer sarcomeres, that is less shortening and were more tender than steaks from 'conventionally chilled' sides (Table 1).

No temperature profiles are given but it is clear that their 'conventional chilling' is quite fast. The authors do not give the mean weights of the sides nor the temperature attained in the conventionally chilled loin steaks at 5h. However a calculation using the graphs of Bailey and Cox (Midas, 1978 also Bailey & Cox, 1976 and Cox & Bailey, 1977) indicates that a regime of air at  $-7^{\circ}\text{C}$ , speed 1 metre/second applied to a heavy side (180kg) would produce a temperature of about  $15^{\circ}$  in the centre loin at 5 h *post mortem* and  $3^{\circ}\text{C}$  at 10 h *post mortem*. Cooling at this rate would fail Bendall's criterion for slow cooling. So on the assumptions made, the control in this report would seem to be 'fast cooled' *m. longissimus dorsi* muscle.

TABLE 1

Tenderness of Beef *M. longissimus dorsi* as Affected by Chilling Method. Selected Results from Bowling *et al.* (1987)

Test	Chilling Method		Significance
	Conventional	Rapid	
Sarcomeres, $\mu\text{m}$	1.93	2.00	$p < 0.01$
Shear force, $\text{kg}^1$	5.03	4.26	$p < 0.01$
Tenderness <sup>2</sup>	4.69	5.30	$p < 0.01$

<sup>1</sup>1.27 cm core.

<sup>2</sup>By panel, 1 = extremely tough, 8 = extremely tender.

The rapid chilled steak was more tender and had slightly less shortening. These findings upset the assumption that faster chilling always means tougher beef.

Sheridan (1990) chilled lamb carcasses 'ultra-rapidly', under air at  $-20^{\circ}$ , 1.5m/s, for 3.5h followed by ageing for 7 days. At 3 h *post mortem* the striploin centre had attained  $+1^{\circ}\text{C}$ . The controls were chilled under air at  $4^{\circ}$ , 0.2 m/s, for 24 h, and at 3 h *post mortem* the striploin centre had attained  $15^{\circ}$ , while by 10h the striploin centre had fallen well below  $10^{\circ}\text{C}$ , to  $5^{\circ}\text{C}$ . These controls had broken the 10 and 10 rule and could be described as 'fast chilled'. After 7 days storage the striploin meat from the ultra-rapid chilled carcasses was as tender as that from the control carcasses, recording about 25–35N shear force in strips of 1 cm square cross section.

This author comments that tender (striploins) may be produced by this ultra-rapid chilling procedure. The 3.5h period is important, since lamb chilled for 3 h, not 3.5 h, at  $-20^{\circ}\text{C}$  was slightly tougher. This points up the precise control that appears to be necessary in VFC specifications.

'Fast' chilling would also be exemplified by the findings of Taylor *et al.* (1972), in whose experiments lamb carcasses were chilled under air at  $-2^{\circ}\text{C}$ , 3m/s, reaching  $10^{\circ}\text{C}$  in the striploin in about 2.5 h and  $0^{\circ}\text{C}$  in 6.5 h; the meat was aged to 5 days and although texture was improved by conditioning (ageing) the fast chilled samples remained undesirably tough.

It appears that the rule of thumb proposed by Bendall over twenty years ago is susceptible of refinement. Further, a more detailed explanation of current findings in terms of muscle biochemistry would be desirable. Jaime *et al.* (1992) excised lamb striploin *pre-rigor* and brought the muscles to target temperatures of  $0^{\circ}\text{C}$ ,  $4^{\circ}\text{C}$ ,  $10^{\circ}\text{C}$ ,  $15^{\circ}\text{C}$ ,  $20^{\circ}\text{C}$  and  $36^{\circ}\text{C}$  within 3 to 4 h *post mortem*. The meat was then aged for 7 days at  $4^{\circ}\text{C}$ .

Taste panel tests show a tenderness maximum in the middle range of target temperatures,  $10^{\circ}\text{C}$ ,  $15^{\circ}\text{C}$ ,  $20^{\circ}\text{C}$ . At  $4^{\circ}\text{C}$  and at  $36^{\circ}\text{C}$  toughness maxima were found.

However in the meat very fast chilled to  $0^{\circ}\text{C}$  a second tenderness maximum was found. A high tenderness sensory score was obtained at 1 day and it had increased at 7 days *post mortem*. Sarcomeres were shorter, the pH was slightly but significantly higher.

The team investigated certain biochemical parameters in the muscles. There was a greater proteolysis, shown by the appearance of more 30 kD molecular weight protein breakdown product in the  $0^{\circ}$  samples. The concentration of free  $\text{Ca}^{++}$  ions in the  $0^{\circ}\text{C}$  muscle fibre sarcoplasm at 5 h *post mortem* was twice as high as in  $4^{\circ}\text{C}$  muscle but 150 times higher than the level in  $15^{\circ}\text{C}$  muscle.

The authors suggest that VFC to  $0^{\circ}\text{C}$  releases extra  $\text{Ca}^{++}$  from the sarcoplasmic reticulum in the microstructure of muscle.  $\text{Ca}^{++}$  is known to stimulate the action of proteolytic enzymes, the calpains (Dransfield, 1994) that produce an 'intense tenderisation capable of overcoming toughness caused by cold shortening'. Proteolysis would be further stimulated by a higher pH at rigor onset.

The hypothesis proposed to explain the increased tenderness results obtained under VFC is minimal and attractive.  $\text{Ca}^{++}$  are the trigger of contraction. Their release from the sarcoplasmic reticulum is in life initiated by a peak of electrical depolarisation entering the muscle fibre from the local motor nerve ending. Their release and rise in concentration from  $0.1\ \mu\text{M}$  one hundred fold to  $10\ \mu\text{M}$  saturates troponin C with calcium. This causes a configurational change, so the inhibitory protein, troponin I, no longer prevents actin in the thin filaments from interacting with MgATP on the heads of the myosin molecule. Contraction follows (Lawrie, 1985) The increase in free calcium ions would tend to increase toughness by provoking shortening. If however the action of proteases were sufficiently fast and widespread the superimposed effect of contraction might be to pull apart sections of muscle, exposing more protein chains to proteolytic attack and perhaps helping to enhance tenderness.

Contraction would require to be continuous along a muscle length in order to increase toughness. In contrast, a small number of breaks created by proteolysis could produce an increase in tenderness out of all proportion to their number. A chain is only as strong as its weakest link. The presence of cracks and breaks in the cytoskeletal structure would focus strain lines when stress is applied and thus propagate fractures (Gordon, 1973).

Very Fast Chilling is worthy of investigation as a technique for improving abattoir process efficiency. It must be designed and operated to ensure tenderness in the prime steak/roasts muscles. There is a great deal of research required to determine the conditions under which VFC can be profitably exploited. Successive stages of breeding and husbandry practice may contribute to variation in the efficacy of VFC effects on meat quality. Major examination and improvement will be needed in the 24 hours before and after slaughter. The welfare of incoming livestock must be paramount to minimize animal distress. The role of electrical stimulation in helping or hindering VFC-induced proteolytic action must be worked out. Hygiene will be crucial in determining whether hot cutting can be installed. The appearance of new strains of spoilage organisms under VFC may present difficulties. Chilling technique itself must be monitored to ensure that precise schedules are followed. Ageing of only prime cuts will need less working capital, but a more controlled and managed post mortem processing, with systematic on-line data accumulation will require higher fixed capital investment.

### CONCERTED ACTION

The results that follow are drawn from the work of the scientists in the European Union Concerted Action 'Very Fast Chilling in Beef' (VFC). There are about fifty scientists in the Union and beyond who are corresponding members of the Action. Their contributions divide into four loosely organised groups:

1. Perimortem, dealing with the periods just before, during and just after slaughter (chair, Honikel); in effect with stress, slaughter and electrical stimulation, their effects on animal metabolism and subsequently on meat quality;
2. Engineering, (joint chairs Gigiel and Daudin) dealing with assessment and accumulation of accurate chilling data and devising engineering protocols to deliver VFC;
3. Biology, (joint chairs Roncales and Dransfield) covering the biochemistry, biophysics and microscopy of muscle-meat, to clarify the effects of VFC at cellular level and to try to relate this to gross properties including tenderness;
4. Quality, (chair Touraille) comprising work on the palatability and also the microbiology of very fast chilled meat.

The Concerted Action has adopted a working definition of VFC, that it is achieved when muscle temperature is brought to  $-1^{\circ}\text{C}$  within 5 h of stunning. This is based on the paper from Bowling *et al.* (1987). We are aware that this is not likely to be a final or exhaustive definition. Tolerances must be researched and advised. Temperature gradients form during chilling and subsequent tenderness results are often difficult to interpret.

Our Engineering group, drawn from several Institutes and meeting at the outset of the Concerted Action have recommended that muscle should be hot boned or hot cut (bone-in) before chilling to try to regulate more efficiently the temperature gradients. A beef side will present some very steep temperature gradients, the faster the chilling the steeper they will be. In all studies temperature fall should be monitored at representative sites in the muscle sample, the better to interpret the subsequent biochemical and tenderness results.

Augustini, (Kulmbach) has studied the effects on meat quality of low voltage electrical stimulation (ES) applied for 15–45 sec. He examined *M. longissimus dorsi* from slaughter

bulls of the Schwarzbunt and Blond d'Aquitaine-Braunvieh cross breeds. Post-mortem pH fell faster in the cross breeds; the difference was maintained in the faster fall under ES. Glycolysis was faster in the crosses. Meat firmness decreased in both breeds under increasing duration of stimulation; no breed difference was found. Shear force was greater in the crosses. Under ES shear force was reduced and breed differences disappeared. Meat colour was influenced by breed and ES, cross bred meat being brighter and redder. ES increased brightness and redness in both breeds, so did ageing from 3 d to 14 d *post-mortem* and the breed difference was consistent. The author comments that genetic influences must be taken into consideration in studying the effects of VFC, both with and without ES. The design of VFC protocols which are likely to be very precise must provide for inherent genotypic variations in metabolism to reduce variability and obtain a consistent high quality in the final product.

Jensen and colleagues at the Danish Meat Research Institute have designed and built a stepwise controlled chilling process. This is an advance in the engineering of chilling that will be used to provide the more precise conditions required for effective VFC.

Beef sides are exposed to air streams through textile windsocks that give an even air distribution, in contrast to the traditional chilling used in large abattoirs. The air temperature and velocity could be controlled. The experimental control was an existing chiller in which the temperature in the *M. longissimus dorsi* fell below 10°C at about 8 hours. Shear force measured in 1 cm square strips from the *M. longissimus dorsi* was lower in the young bulls from the controlled process where the temperature was kept above 10°C for 12 h or more; tenderness improvement was detected by sensory panel only after 14 h above 10°C. The authors comment that animal to animal differences in tenderness were bigger than differences in average values for the two chilling techniques; use of the controlled process will improve tenderness on average. Drumm *et al.* (1992b) found a weak relation between temperature at 10 h and tenderness, and refer to Lochner *et al.* (1980) and Marsh *et al.* (1981) who proposed that maintaining temperature, of about 37°C, in the very early period *post mortem*, the first 3 h, was critical for tenderness, but this seems to conflict with Jaime *et al.* (1992); lamb muscle held at 36°C gave tougher meat.

Jensen has investigated pre-slaughter stress control of cattle in Denmark. Her data show that the incidence of high ultimate pH, defined as > 5.8, can be reduced if the farm-abattoir journey is short and careful, and if slaughter is the same day, ideally on arrival. Farmers, drivers and lairage must all cooperate in scheduling. Her checklist of pre-slaughter care starts far back with dehorning of calves. Other points are that cattle should move in their production groups, and be loaded directly from stalls into the lorry, using ramps. The abattoir lairage must be carefully designed to minimize stress. Animals must be handled with great care and consideration. We are concerned in the VFC work that animal to animal metabolic variation be as small as possible and in any event humane considerations must be uppermost.

Kennedy of the University of Leeds, (formerly York) and Jones and Miller (ETG Air Products plc, Basingstoke) built a mobile chiller refrigerated by liquid nitrogen so ambient can be held at -60°C. It can be set to bring the centre striploin temperatures in up to 17 beef hindquarters down to 1°C within 4, 6 or 8h. Preliminary trials by the team had found that very fast chilled hot boned sirloin (*M. longissimus dorsi*) was as tender as slow chilled controls, wherein no part of any muscle was taken below 10°C within 10 h of stunning. Finite element modelling of the chilling of beef hindquarters was developed interactively to fit data obtained and to estimate the air temperatures required.

The team showed that it was possible to obtain the criteria for very fast chilling within the sirloin of the beef hindquarter without hot boning. The fastest chill rates achieved took the centre of the sirloin to 1°C within 4 h with a freeze depth of less than 1cm. The

lowest air temperature used was  $-40^{\circ}\text{C}$  with a heat transfer coefficient of  $40\text{W}/\text{m}^2\text{K}$ ; lower temperatures would be required if air velocity were lower. The team comment that their finite element model fits the data reasonably well. Heat transfer will vary due to the shielding of internal surfaces by the carcass shape, so perhaps more designed air delivery may be needed. In the mass of the round, above the sirloin as suspended, about three quarters of the meat remained above  $10^{\circ}$  for 10 h even though the sirloin had undergone VFC.

Cuthbertson and Owen of the Meat and Livestock Commission, UK, worked with Jones, Miller and Kennedy. They used the mobile fast chiller in trials to evaluate the relation between chill rate and eating quality. Centre sirloin was brought to  $1^{\circ}\text{C}$  within 4 or 6 or 8 h but the temperature at 1 cm below the surface was not allowed to fall below  $-2^{\circ}\text{C}$ , that is, freezing. The team achieved a VFC target of  $1^{\circ}\text{C}$  within 4 h in the sirloin without recourse to hot boning. Sensory tests have indicated that VFC meat is as tender as controls but that there is a great deal of variability. Controls were comparable sides wherein the centre sirloin was not chilled below  $10^{\circ}\text{C}$  in 10 h post stunning.

Gigiel of the University of Bristol has examined current cooling rates and weight loss in 14 beef chillers in UK commercial abattoirs. Sides were removed from the chillers at 24 to 48 h post mortem. In only 4 of the chillers was an average temperature of  $7^{\circ}\text{C}$  or less found in the deep leg (centre round). Weight loss was 1.1%–2% at 24 h and 1.55–2.3% at 48h; its cost was 20 times the cost of energy used in chilling. The author comments that an important contributor to weight loss variation was the different time span between slaughter and hot weighing. Chillers should cool effectively, minimise weight loss and energy use; design and operation are often short of the ideal. Air temperature in the first 24 h must be low, so held by sufficient plant capacity, while air velocity over the sides must be above the minimum required. The study points up the difficulties in obtaining chilling conditions that meet the operational criteria in batch chilling (Drumm *et al.*, 1992a). Proper process control is not achieved in batch chilling, whether intended to effect 'VFC' or to effect controlled slow chilling.

Roncalés and Beltran of the University of Zaragoza have extended their lamb investigations to beef muscles. Hot boned *M. sternomandibularis* was brought to  $0^{\circ}\text{C}$  in 2 h, under air at  $-70^{\circ}\text{C}$ . A control was stored at  $-1^{\circ}\text{C}$ . Both samples showed sarcomere shortening to mean values of  $1.58\ \mu\text{M}$ , VFC, and  $1.64\ \mu\text{M}$ , control. Calcium ion release was higher in the VFC meat at 5 h, but after 14 d free calcium levels in both treatments had fallen considerably. SDS gel electrophoresis showed that a greater protein breakdown had occurred in the VFC treatment.

The team reported later work to the Concerted Action in April, 1996. They restrained the beef *M. sternomandibularis* samples by fixing both ends to boards, and chilled either in glycol at  $-6^{\circ}\text{C}$  (VFC) or in a chill room at  $-1^{\circ}\text{C}$  (control). The VFC samples took 2.5 h to reach  $0^{\circ}\text{C}$ , the control took 20 h. Ultimate pH was 5.75 in VFC muscle, 5.60 in controls, sarcomere length ( $\mu\text{M}$ ) was 1.72 (VFC) and 1.70 (control). Taste panelling found VFC meat to be more tender, at 2.5 than control meat at 1.5, on a scale of 1 = extremely tough, 9 = extremely tender. Both treatments were tough as *M. sternomandibularis* has high background toughness due to high connective tissue, but the interest in these results lies in the higher score found for the VFC muscle. More  $\text{Ca}^{++}$  ions were released to the myofibrillar space in VFC conditions. The activity of  $\mu$ -calpain decreased very fast in VFC samples and the authors comment that this indicates a more intense activity by this enzyme. m-Calpain and calpastatin, the inhibitor, showed no difference between treatments. Electrophoretic patterns showed a more intense degradation activity in VFC samples as early as 2.5 h post mortem, when  $0^{\circ}$  was reached. This was shown by the appearance of a 30 kD polypeptide band and also by another band with a molecular weight immediately lower than that of myosin fraction LC1 and troponin I; the significance of this is not clear but strengthens the evidence for enhanced proteolysis under VFC.

The authors comment that although *M. sternomandibularis* is convenient for demonstrating biochemical parameters and shows that VFC can induce tenderisation the high background toughness is discouraging. The VFC condition, 0° in 4h maximum is critical. They are optimistic 'there is enough biochemical evidence that the benefits of VFC in terms of beef tenderness can be obtained.'

Steen, Claeys and Demeyer, University of Ghent, Belgium chilled excised beef *M. longissimus thoracis* in an ice bath at 1.5h post mortem. Controls were unexcised chilled conventionally from the alternate side. The VFC treatment muscle attained 0°C in 4–5h, the control had reached 20°C. Rate of pH fall and final pH was not affected. Cold shortening occurred in the VFC muscle and at 1d pm free calcium concentration was higher.

This team reported further work, with Buysse, last April. More detailed temperature measurements in cooling sides showed that considerable differences in temperature can arise in different muscles of the same side.

The cooling profile must be specific to obtain 0°C within 3–5h for VFC and this cannot be obtained in several muscles simultaneously. Even when carcasses are hot cut after dressing, to secure similar cooling conditions in all muscles will be difficult. The group conclude that VFC of isolated beef muscles toughens them. In some experiments evidence was obtained for increased protein fragmentation associated with an increase of free Ca<sup>++</sup> and increased  $\mu$ -calpain activity. Tenderising effects seem to be overcome by cold-shortening.

O'Mahony working in the National Food Centre, Dublin, Ireland, with myself, and McKenna of University College Dublin has applied VFC to *M. longissimus lumborum* (LD) from rib 11 to the pelvic bone. The work has been replicated in 20 Hereford cross heifers. After conventional slaughter and dressing, the muscles were hot cut and left on the bone. One side's muscle was cut across transversely and the two halves, anterior and posterior, assigned to VFC or control. The other side's halves were assigned oppositely, to control or VFC. The VFC halves were chilled under air at -27°, 2m/s, and attained 0°C in 4h. they were held at -1°C to 48h. The control halves were held at ambient (c.15°C) for 24 h and then chilled at 0°C for a further 24 h. They reached c. 20°C at 10 h and cooled to 2°C in a further 12 h. Tenderness was measured using the Warner-Bratzler shear on 1.25 cm cores. VFC steaks were tougher ( $p < .05$ ) at 2d recording about 97N, while controls recorded 76N, but at 7d both had decreased to about 63N and at 14 d were about 55N. This would be rated 'tender' by a sensory panel (Joseph & Connolly, 1979). It had been thought that cold toughened beef would not tenderise as much as slow cooled beef (Joseph & Connolly, 1977) but these results call this general observation in question.

O'Mahony subsequently examined intermediate rates of cooling categorised as Fast Cool 1 and Fast Cool 2. The sections of muscle achieved 4°C and 8°C in 5 h postmortem. Both treatments yielded meat which was tough, about 150N, at 2 d. This value fell to 120N at 7d and 100N at 14d. The contrast between these 'fast cooled' treatments and both 'slow cooled' and VFC in tenderness was remarkable, and emphasises once more the difficulty of obtaining exactly the right conditions whereunder VFC will not toughen meat. There was evidence of crust freezing in the meat and the authors consider that this plus the skeletal restraint may have played a part in improving tenderness.

Sheridan, McGeehin and Butler, working in the NFC, Dublin, and University College Dublin, continued work on Lamb, begun by Sheridan (1990). Carcass temperature was taken in the deep round. A VFC regime produced loins as tender as those from conventionally chilled carcasses. Electrical stimulation improved tenderness at 1d post mortem but by 5d it made no difference.

White, Troy and McKenna (also of the NFC and UCD) chilled hot cut and vac-packed *M. longissimus lumborum* in brine to reach -0.2°C by 4.5 h post mortem. After sampling at

5h all samples were repacked and placed in an incubator at 15°C for 12h. ('equilibration') Control samples were slow chilled to reach 15°C by c. 15 h pm. Subsequent aging to 7d was at 4°C. The VFC with equilibration samples were significantly tougher ( $p < 0.001$ ) by both sensory analysis and by Warner Bratzler shear, on 1.25 cm cores, at both 1d and 7d *post mortem*. VFC samples sheared at 12.3 kg, controls at 7.45 kg at 1d post mortem, and at 10.46 kg and 4.72 kg at 7d. There was severe cold-shortening in the VFC samples and pH fell more slowly, being 0.3 unit higher at 6h pm. SDS PAGE showed a similar intensity of the 30kDa band in both VFC and control samples.

These authors comment on the absence of crust freezing in their brine chilled VFC samples and suggest that crust freezing and the restraint it imposes is critical in ensuring that VFC meat will be tender.

Kiely in Meadow Meats of Rathdowney, Ireland investigated the use of an industrial chill for very fast chilling. Segments of striploin were exposed to blast chill conditions, -25°C under 8 metres/sec air speed. Meat temperature at 2.5 h was 6° to 7°C. At 14d the meat was slightly tough. Striploin stored at lower air speeds, 1m/s, and higher temperatures, -20°C, achieved 16°C in 3 h and was subsequently slightly tender to tender. This team concluded that VFC conditions could not be easily achieved in factory chills at present, even under full power.

Taylor, Richardson and Perry of the University of Bristol, U.K., have investigated the effects of VFC on *M. sternomandibularis*. They observe that this muscle is small enough to be cooled easily within 5 h of slaughter by holding at 1°C. In larger muscles temperature gradients will be set up by VFC conditions and so some cold toughening due to shortening may occur, confusing the overall result. Paired muscles were removed from 12 beef carcasses and chilled at 1°C for 24 h, VFC, and 16°C for 18 h, 1°C for 6 h, control. Pronounced effects were found attributable to cold-shortening, muscle length was halved and sarcomeres were too short to be measured. Texture, however, did not reflect this. In 1×1×2cm strips they recorded shear force of 14.9 kg at 2d falling to 9.8 at 8d in VFC meat, while in control meat the shear force values were 12.6 and 8.5 kg. The variability was greater in VFC meat. The shear force values are higher than those usually found in *M. longissimus dorsi* but *M. sternomandibularis* contains much more connective tissue. The tenderising from 2d to 8d of about 4 kg is similar in both test and control.

Much interest focusses on the mechanisms of tenderising in these studies. Zamora and Dransfield at the INRA Meat Research Station, Theix, France have studied the calpains and their inhibitors, the calpastatins, in very fast and slowly chilled muscles. *M. longissimus dorsi* from 4 17-month Charolais bulls was removed at 1h post mortem and cut across into 5 cm slices. 4 slices were held at 15°C for 24 h, then held at 4°C for 6d, these were controls. Another 4 were stored at 0°C; these were VFC. Calpain and calpastatin activities were measured at 1 h, 1, 2, and 6d post mortem in controls and 8 h, 1, 2 and 6d in VFC. Toughness was measured at 2 and 7d.

$\mu$ -calpain, which is catalysed by micromolar calcium concentrations, and m-calpain, requiring millimolar concentrations were both measured. There were no significant differences in the concentrations in the two chilling treatments, and both showed a decline so that by 6d  $\mu$ -calpain had disappeared while m-calpain fell to about half its starting level. Calpastatin, the inhibitor, slightly but not significantly higher in the vfc meat, fell to about half its starting levels by 6d and no significant difference was found between the chilling treatments. Toughness tenderness was measured in 1x1cm square strips. VFC meat had a mean shear force of 240N/cm<sup>2</sup> at 2d; this was significantly higher than the control meat at 160N/cm<sup>2</sup>. By 6d both shear force values had fallen, to about 190 and 120 N/cm<sup>2</sup> respectively but the variation was very high and the means were not significantly different. The authors comment that meat stored at 0°C was tougher than that stored at 15°C and this may be due to the rapid inactivation of the calpains and/or



increased calpastatin in the rapidly chilled meat. There is a great deal of variability in the meat samples.

Another team at INRA, Theix, Santé, Lacourt and Le Pottier, have studied the effects of cooling rates on myosin  $\text{Ca}^{++}$  ATPase. Myosin is the muscle protein present in the greatest quantity and its denaturation will substantially affect water holding capacity and thereby texture and sensory response. The work was carried out on turkey breast muscle and it was shown that lowering muscle temperature as quickly as possible reduced the denaturation of myosin.

Lepetit at Theix, has investigated rigor strength and temperature in rabbit *M. longissimus dorsi*. Critical questions are 'when does tenderisation start?' and 'what is the initial resistance of the structure on which the ageing process acts?' Proteolysis begins soon after slaughter according to Troy *et al.* (1986); Dransfield (1992) suggests that tenderisation starts when pH drops below 6.1 at 2–7 h pm and this activates calpain. Of course it might be suggested that a continual low level degradation and replacement of protein structures is continuing throughout life in all tissues and this process is accelerated postmortem.

The work showed that the resistance of muscle fibres began at about 0.05N/sq cm and during rigor could increase 40 fold. Resistance at rigor onset is maximal if the muscle is stored at 10°C–15°C. Changes in rigor strength at fixed sarcomere length can only be explained by biochemical changes in bonds. The low strengths obtained at low temperatures are explained by active tenderisation proceeding before full rigor strength has been achieved.

The data are consistent with tenderisation starting before completion of rigor and with a variable rigor strength.

Trevisani, Loschi and Severini at the University of Perugia have studied the effects of very fast chilling on unrestrained *M. sternomandibularis*. Severe cold-shortening occurred and the meat was extremely tough; the team suggest that this muscle high in connective tissue may not be a good model for VFC studies.

At the Swedish Meat Research Institute, Wahlgren and Tornberg have examined the effect of VFC on muscle shortening, isometric tension and tenderness, using *M. longissimus dorsi* and *M. semimembranosus* from Swedish Lowland bulls. VFC was defined as 0°C in 4h post mortem in the muscle centre. They showed that fast cooling simulating the fastest possible chilling for hot-boned LD and SM muscles without surface freezing resulted in shortening of 25% and this produced unacceptable tenderness in LD but not in the SM muscle. VFC applied to the hot boned LD will result in tougher meat if shortening is not hindered structurally by, for example, freezing. They predict that their cooling regime is close to the optimum for SM but faster cooling would give tougher meat.

The Concerted Action has included work on the microbiology of VFC meat; it is important that no bacterial hazard is overlooked in designing precise and very fast chilling regimes. Hebraud and Michel at Theix have studied the effect of VFC on the lag phase, growth and protein synthesis in the psychrotrophic spoilage bacterium, *Pseudomonas fragii*. Cold shock was induced by transferring cultures from 20°C or 30°C to 5°C; the downshift was followed by a lag phase of 3 h or 5 h, respectively. After the lag, growth resumed with a 7h generation time, as is usual in steady state cultures at 5°C. Most of the relative rates of protein synthesis did not change after the stress. The analysis of protein patterns displayed the over-expression of 16, and 24, proteins following the 20°C to 5°C and 30°C to 5°C shifts; the two shifts shared similar variations for the synthesis of 20 proteins. The main 'cold-shock proteins' (Csps) of *P. fragi* were four low molecular weight proteins, both belonging to the family of low MW nucleic acid binding proteins such as CspA, the major *Escherichia coli* CSP. These four proteins play a major role in the adaptive response of *P. fragi* to very fast chilling.

Touraille of INRA who leads our Quality Group, which includes microbiological work,

has drawn attention to the need for very precise and comprehensive recording of every variable involved in experimental work on VFC. The biochemical mechanisms underlying texture in muscle are seen to be extremely complex and interactive, as (for example) Dransfield has shown. Variables may be listed as (1) species (2) breed (3) anatomical muscle site, together with (4) chilling rate measured so to be truly representative (5) degree of stretch and resultant shortening assessed by sarcomere and whole muscle length (6) pH at stated position and times post mortem (7) if electrical stimulation is used all details of current, time and mode of application must be given (8) duration of ageing must be quoted.

Texture evaluation must give details of sample size, cooking, physical or sensoric technique; finally and crucially the treatment of the control or reference to which the very fast chilled meat is compared must be as equally well described. The age of the animal may be critical in VFC work since there is reason to believe that the amount of protein synthesising and protein autolyzing enzymes varies with age, both being maximal where growth is proceeding rapidly.

## CONCLUSIONS

Dransfield has described the importance of the rate of rigor mortis development in meat quality; it is the most important single factor affecting ageing and meat texture. The rate is affected by genetic differences, by pre-slaughter transport and handling and by chilling rate *post mortem*. Too rapid chilling while pH remains high often toughens meat but allowing rigor to proceed just below the freezing point,  $-2^{\circ}\text{C}$ , produces tender meat. The rate of rigor development in pre-rigor frozen meat during thawing can be varied by varying thawing rate to produce a range of textures.

Variability in rigor rate affects final meat quality and processing systems must be found to reduce variability; very fast chilling alone may simply exacerbate the effects of rigor variability. It is suggested that the rate be monitored post mortem, that electrical stimulation be applied only to carcasses entering rigor slowly and then very fast chilling for consistently tender meat could be implemented.

The working definition of Very Fast Chilling is the attainment of  $-1^{\circ}\text{C}$  at 5 h post mortem. This necessitates air temperatures well below  $0^{\circ}\text{C}$  and so temperature gradients develop in the muscle-meat. Profound biochemical changes are induced by cooling pre-rigor muscle from all species examined, and these are especially sensitive to temperature changes in the region  $5^{\circ}\text{C}$  to  $-5^{\circ}\text{C}$ . Fresh muscle held between  $0^{\circ}\text{C}$  and  $5^{\circ}\text{C}$  will produce tough meat compared to muscle held at  $10^{\circ}\text{C}$  to  $20^{\circ}\text{C}$ . Muscle held between  $-1^{\circ}\text{C}$  and  $-5^{\circ}\text{C}$  will produce quite tender meat in a few days; it will be acceptably tender after ageing for up to 3 weeks.

Variability is a serious problem in VFC meat and this is attributed firstly, to the steep temperature gradients induced in muscle by very fast chilling.

Calcium ions are released under VFC in pre-rigor muscle and these stimulate proteolysis leading to tenderisation. If this is to be exploited industrially in particular prime muscles then a better understanding of the biochemistry, biophysics and structure of muscle-meat is essential. At present VFC is not ready for full scale industrial use. Pilot plant studies should be undertaken based on models proposed in the present paper.

There is evidence that VFC in carefully defined circumstances can produce tender beef. The mechanism of tenderising must be determined. Does it depend on crust freezing restraining shortening, or vigorous proteolysis overcoming cold shortening and toughening?

The Concerted Action teams have produced some novel and exciting results. It may be that the introduction of VFC with its potential for same day processing could transform the Beef Industry in a way not seen since refrigeration was brought in a century ago.

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