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Milestone

Characterisation of temperature profiles (PSC and RT phases) of VFC for chilled and frozen product with commercial systems VFC trials completed.

Milestone report detailing trial outcomes and screenshots of freeze/thaw and chilling trials to be submitted and accepted

Abstract

- An experiment was conducted at HW Greenham & Sons, Tongala on December 8-9 2009.
- This experiment aimed to compare the quality of meat subjected to a very fast chilling profile with a conventional chilling profile for chilled and frozen product.
- There were 4 treatments: conventional chill (CC), very fast chill (VFC), conventional freeze (CF) and very fast freeze (FF).
- Strip loins from 12 animals were randomly allocated to one of 2 chilling treatments (CC or VFC). Strip loins from 8 animals were randomly allocated to one of 2 freezing treatments (CF or FF).
- A range of measurements were taken including shear force, drip loss and colour stability measurements, temperature, pH and sarcomere length.
- The major finding was that cooling rate had little effect on the factors measured. The phase (chill or frozen) endpoint influenced results for colour and shear force.
- Sarcomere length was similar in all treatments so could not account for differences between treatments for shear force.
- The shear force values for frozen loins were similar on day 1 to the values for chilled samples on day 10, but did not reduce any further between days 1 and 10.
- Frozen samples were darker and browner in colour than chilled samples.
- Fast chilled samples had slightly lower values for fluid loss measured in the bag after processing than slow chilled samples.

Project objectives

1. Verification of Smartchill specification for hot boned beef
2. Characterisation of temperature profiles of Smartchill for chilled and frozen product with commercial systems

Success in achieving milestone

The experiment in this milestone was conducted as planned. The results provide new information that will assist with defining the mechanism of tenderisation due to VFC and the chilling profile required to undertake successful VFC for chilled and frozen product.

Recommendations

- Further research and development should be considered before VFC can be commercialised.
- Practical implications involved with gaining efficiency from improving the rate of throughput of product through refrigeration systems should be considered in developing applications for VFC.
- Potential exists to use very fast chilling to prevent sarcomere shortening without the need for electrical stimulation on the slaughter floor. For this reason further investigation is warranted for the purpose of improving water handling capacity and functionality of grinding beef.
- Definition of the critical parts of the phase change process (liquid to solid) should be a priority in any future work designed to elucidate the mechanism for “accelerated tenderisation” with VFC.

Appendices: Experiment 3 report

Background

This report describes the third of three experiments conducted on site at HW Greenham & Son, Tongala Victoria. These experiments used hot boned beef cooled with a plate freezer. The purpose of this study was to provide proof of concept for very fast chilling in beef, using a commercially available cooling system.

The first experiment established that very fast chilling was feasible if the carton type and plate spacing's were changed. The magnitude of the spacing between the plates required to achieve very fast chilling was smaller than contemporary spacing's, in the order of 60mm, and the carton needed to be made from solid fibre board rather than fluted cardboard.

The second experiment tested the quality of beef strip loin chilled with this smaller spacing but the results were potentially compromised by a power failure that occurred during the experiment.

The third experiment aimed to repeat the second experiment without the power failure, and the design was expanded to include frozen as well as chilled product in accordance with milestone requirements.

As well as providing proof of concept, VFC has potential applications for both chilled and frozen meat product lines produced at Tongala if successful.

Aim

To compare the quality of meat chilled with a very fast temperature profile and a contemporary temperature profile for chilled and frozen product.

Method*Experimental design*

The design consisted of 2 physical phase endpoints (phase = chilled or frozen) and 2 rates of chilling (rate = slow or fast). This provided 4 treatments (Table 1):

Table 1 Treatment names and descriptions

Phase	Cooling rate	
	Slow	Fast
Chilled	Conventional chilled (CC)	Very fast chilled (VFC)
Frozen	Conventional frozen (CF)	Fast frozen (FF)

20 carcasses were used for the experiment. Carcasses were allocated to treatments in blocks of 4 because the very fast chilled cartons contained 4 loins each. Dummy loins that were similar in physical dimensions to the loins measured were used to pack the CC and CF cartons as these required 8 loins to fill to capacity. Two blocks of 4 carcasses (8 carcasses) were allocated to frozen treatments and three blocks of 4 carcasses (12 carcasses) were allocated to chilled treatments. Initially it was intended that 12 carcasses would be allocated to frozen treatments as well but availability of cows on the day restricted the availability of loins of a consistent type and limited the numbers allocated to the frozen treatments.

Within each block of 4 carcasses, left and right loins from each carcass were randomly allocated to either very fast or conventional chilling rates such that 12 loins were allocated to VFC, 12 to CC, 8 to FF and 8 to CF.

Refrigeration systems

Loins subjected to CC were placed in plastic crates and chilled in a conventional blast chiller for 21 h post mortem. Loins subjected to VFC were placed in solid fibre cartons, and cooled in a plate freezer with a spacing of 60mm until the internal

temperature of the loin fell below 0°C and stabilised in time (2-3h). At this time the cartons containing the VFC loins were placed into the same blast chiller containing the CC loins and kept there for about 18h. Loins subjected to CF were packed in a conventional fluted cardboard carton, then placed into a plate freezer with a spacing of 120mm for 24h and then into a blast freezer. Loins subjected to FF were placed in a solid fibre carton and in a plate freezer with a spacing of 60mm for 24h and then into the blast freezer for storage before transport and processing.

Chilled loins were transported from the abattoir to the laboratory in an esky with wet ice (0°C), and then stored in a chiller room (2-4°C ambient air temperature) before processing prior to analyses. Frozen loins were transported in an esky containing dry ice (-56°C) and then stored in a chest freezer set at -20°C before processing.

Meat

The meat was strip loin 2140 (Anonymous 2005), hot boned and packed in heat sensitive vacuum sealed plastic bags at the end of the processing chain prior to allocation to cooling treatment. Pre-dressing high voltage electrical stimulation was used on all carcasses. The carcasses were allocated to treatment blocks in the order that they were killed. The carcasses were from 5 different vendor consignments; 11 from consignment A, 6 from consignment B and 1 from consignments C, D and E. Fifteen of the carcasses were from female dairy type animals; two were classified YS (yearling steer), and the remaining three were classified PGS, YGS (young steer) or YPS (Anonymous 2009). The mean hot carcass weight was 197.8±31.5kg.

Measurements

pH

The pH of the loin was measured post slaughter prior to vacuum packaging (pH₀), at 24h post slaughter (pH₂₄) and prior to processing for shear force. On each occasion a mean of 6 measurements was used to estimate pH.

Temperature

For loins in the chilled treatment 2 stainless steel temperature probes (100mm in length), attached to a Hobo data logger, were inserted longitudinally from the caudal end of the loin. One of the probes was inserted 5 mm from the dorsal surface and the other in the centre of the meat. I-button temperature loggers were placed in the all frozen loins and some chilled loins. These were placed in the centre of the loin about 100mm from the caudal end.

Shear force

Shear force was measured using a Lloyd texture metre. Samples were prepared and cooked using the method described by (Perry *et al.* 2001) with a few minor modifications.

1. Samples were cut into cook blocks of approximately 250g at day 1 post slaughter for chilled samples (CC & VFC) and after thawing for frozen samples (CF & FF).
2. Samples were aged for 1 and 10 days post slaughter for chilled samples (CC & VFC) and 1 and 10 days post thaw for frozen samples.
3. For chilled samples (CC & VFC) cook blocks were vacuum packed before ageing and then frozen after ageing at -20°C.
4. Frozen samples (CF & FF) were thawed in a 2°C chiller for 24 h prior to cutting into cook blocks of 250g.
5. Samples were taken from the cranial half of the strip loin (Figure 1) and allocated randomly to ageing periods according to relative cranial or caudal locations within the cranial half.
6. Cook blocks were rested after cooking in a 4°C chiller overnight before testing.
7. 6 subsamples with a cross section of 10mm wide by 10mm deep (1cm²) were prepared and tested from each block. Testing was completed using a Lloyd machine fitted with a Warner Bratzler shear blade set.

Sarcomere length

Sarcomere length was measured using a laser diffraction device. Chilled samples (CC & VFC) were frozen at day 1 post slaughter

Colour stability

Samples measured for colour were packed in vacuum and stored for 10 days at 2°C. They were then cut transversely into strips 30mm thick, over wrapped in polyvinyl chloride cling wrap on black polystyrene trays, stored in a display cabinet at 4°C with lights on and measured daily for 7 days using a Hunterlab minilab LE 45/20.

Water holding capacity

Bag loss, drip loss and cook loss were measured to estimate the effect of treatment on water holding capacity.

Bag loss was the amount of fluid lost from an entire loin whilst in the vacuum bag in which they were packed prior to treatment. For chilled loins, this measurement was made at the plant 16 – 24h after treatment. For frozen loins, the measurement was made after thawing for 48h in a chiller (2-4°C), 6 weeks after treatment.

Drip loss was the amount of fluid lost during ageing calculated as the difference between the weights at day 1 and 10 as a percentage of the weight at day 1. For chilled treatments, the colour block was used (approximately 100g taken from the caudal end of the loin). For frozen treatments, the day 10 shear force block was used (approximately 250g in weight taken from the cranial end of the loin) after the loins had been thawed for 48h at 2-4°C.

Cook loss was the amount of weight calculated as a percentage of the pre-cook weight that was lost during cooking prior to shear force measurement.

Shear force sample 1 250 g	Shear force sample 2 250 g	Colour / Drip Loss 100 g	pH Temperature
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Cranial End

Caudal End

Figure 1: Relative positions within the loin and weights (g) of samples taken for shear force, colour, drip loss, pH and temperature.

Statistical analyses

All statistical analyses were done using Genstat for windows version 12 (VSN International Ltd, 5 The Waterhouse, Waterhouse Street, Hemel Hempstead, HP11ES UK). Carcase was partially confounded with treatment in the sense that carcasses were allocated to either chill or frozen phase endpoints and within these treatments sides within carcasses were allocated to slow or fast cooling rate treatments. Loins used for the chill endpoint were therefore from different carcasses to those used for the frozen endpoint. However sides for slow rate were from the same carcasses as those for the fast rate. This design expedited transfer of loins from slaughter to treatment and commencement of treatment that would have been delayed excessively if carcase sides had been randomized across the 4 treatments. Cooling rate was considered to be of more interest than phase for comparisons of treatments.

Analysis of variance was used to test the effects of treatment on loin weights, linear dimensions, pH, temperature, colour after blooming and sarcomere length with blocking for carcase/side.

A general linear model was tested using REML (residual maximum likelihood) for the shear force and colour data. For shear force, treatment and day were fixed effects and carcase, side, day and sample fitted as random effects. This allowed for variance to change according to day.

For the colour display data the method described in Jacob *et al* (2007) was used. A repeated measures model was fitted with a power correlation between pairs of times and different variances for treatments and display time.

Equation 1 Genstat code for colour analyses

```
"model 2c - -power correlation between pairs of times, different variances"
```

```
vcomp [fixed=Phase*Rate*Time] random = Carcase +  
Carcase.Treat+Carcase.Side.Time ;con=pos
```

```
vstructure [ Carcase.Treat] factor=Treat;model=diag
```

```
vstructure [ Carcase.Side.Time;coord=period]
```

```
factor=Time;model=power;hetero=outside
```

```
reml [print=wald,dev,comp,mean;pse=allesti ;maxcycle=1000] y[1]
```

For bag loss, drip loss and cook loss chilled and frozen samples were analysed separately due to differences in sample size and temperature histories between chilled and frozen samples. Analysis of variance was used to test the effect of chilling rate with blocking for animal/side. Initial weight of sample was used as a covariate.

Results

Physical characteristics

Table 2 Weights (g), linear dimensions (mm) and pH of strip loins for, CC, VFC, CF and FF treatments (values are means)

Parameter	Treatment				LSD	P value
	CC	VFC	CF	FF		
Weight (g)	2489	2491	2550	2430	179.76	0.58
Length (mm)	361.9	363.6	371.5	354	25.32	0.53
Width – cranial end (mm)	129.2	124.6	131.3	130	11.60	0.5
Height – cranial end (mm)	54.13	49.72	51.3	52.6	4.43	0.12
pH ₀	6.18	6.16	6.14	6.2	0.06	0.23
pH ₂₄	5.81 ^a	5.88 ^b	*	*	0.06	0.014
pH _u	5.76	5.78	5.77	5.78	0.04	0.563

*values not measured

pH₀ = pH at the time of vacuum packaging

pH₂₄ = pH 24h post slaughter

pH_u = pH >48H post mortem

Values with different values a, b within a row are different.

There was no effect of treatment on weight or linear dimensions of the loins (Table 2). There was an effect of treatment on pH₂₄ but not pH₀ or pH_u.

The pH₂₄ was higher for VFC than CC. CF and FF was not measured at 24h post-mortem.

Table 3 The temperature (°C) of loins cooled slow or fast in the first 3 hours following packaging (values are means) LSD (P<0.05) for comparing means at the same time = 1.88.

Time (hours) after packaging	Cooling rate	
	Slow	Fast
0	32.36	31.62
1	26.23	19.12
2	17.26	3.36
3	10.9	-1.4

There was no effect of phase on the rate of temperature change in the first 3 hours following packaging so the means comparing cooling rate (includes both phases) only are presented in Table 3. Temperature was significantly different at 1, 2 and 3 hours (P<0.01) and not at 0 hours (P>0.05).

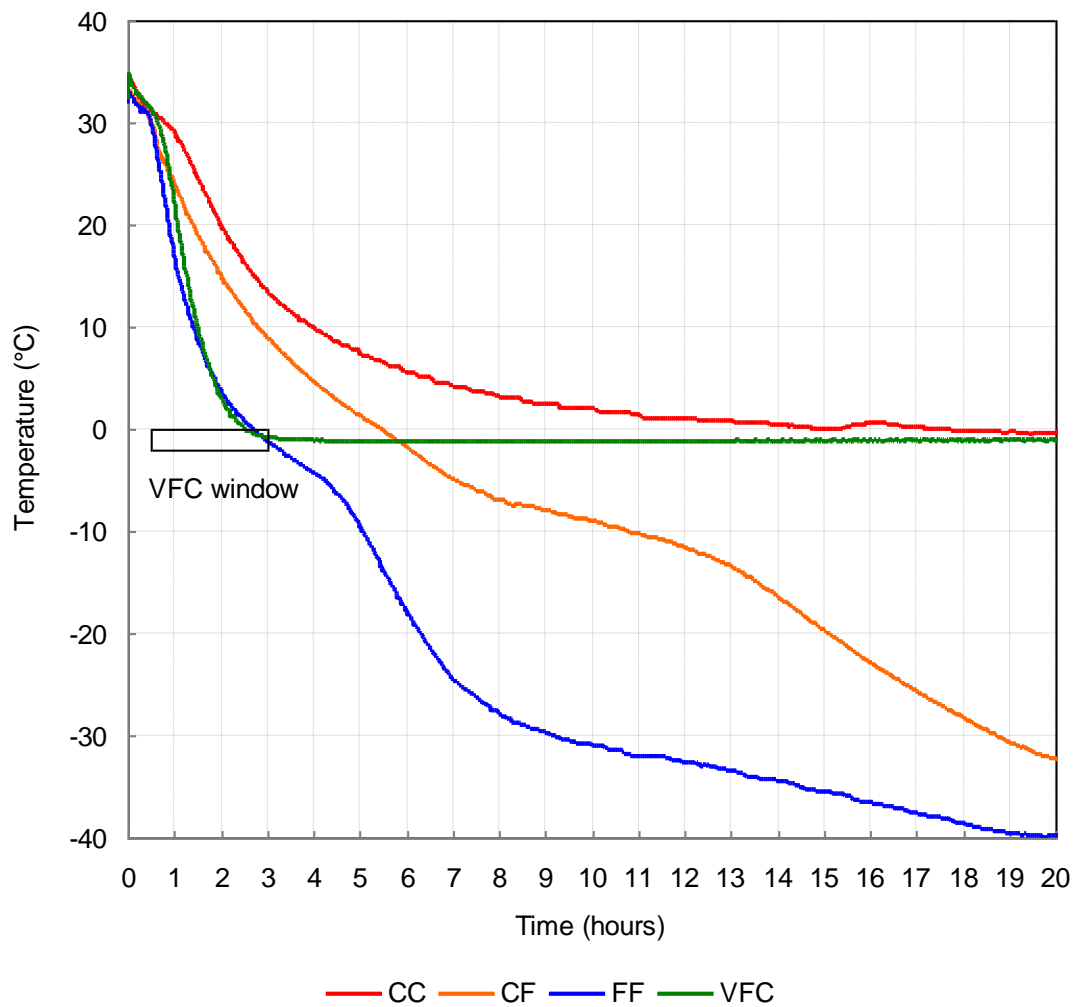


Figure 2 The temperatures measured at intervals of 1 minute for 20 hours post slaughter in CC, VFC, CF and FF loins (values are means, rectangle represents

The profiles of temperature plotted against time were different for different treatments (Figure 2). Minimum temperatures reached within a 20 hour period were -0.78, -1.24, -36.6, and -40.7°C for CC, VFC, CF and FF respectively. The latent heat period appeared to occur at a lower temperature and for a longer period of time in CF compared to FF.

Quality attributes

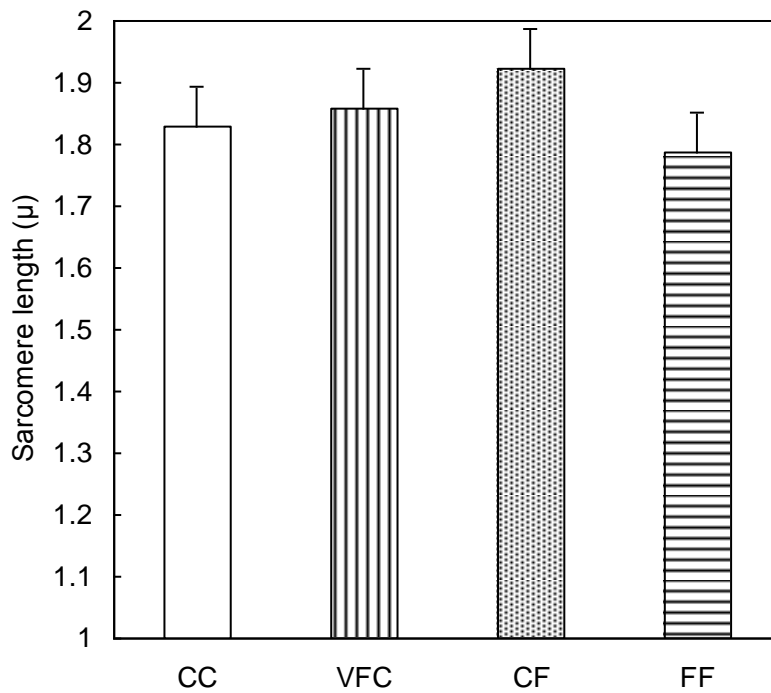


Figure 3 Sarcomere lengths (μ) for CC, VFC, CF and FF (values are means, bars = SEM, $P < 0.05$)

There was no difference ($P > 0.05$) between treatments for sarcomere length (Figure 3). Furthermore sarcomere lengths were longer than the benchmark value for toughening of 1.6μ in all treatment groups.

Table 4 The effect of treatment on colour after cutting and blooming. Values are means, LSD is the least significant difference ($P<0.05$) and are the value for the treatment (phase or rate) that is significant, and in the case of chroma where both are significant it is for phase.

Parameter	Treatment				LSD	P value		
	CC	VFC	CF	FF		Phase	Rate	Phase*Rate
L	28.34	27.81	22.76	21.59	3.8	0.006	0.163	0.576
a	19.98	19.04	22.53	22.3	2.03	0.01	0.16	0.449
b	18.57	17.05	20.03	18.79	0.76	0.171	0.002	0.72
Hue	42.8	41.74	41.53	40.07	0.91	0.147	0.015	0.672
Chroma	27.29	25.57	30.19	29.23	2.3	0.034	0.018	0.504

Colour after cutting and blooming was significantly affected by treatment in a number of ways (Table 4). Phase (chill or frozen) had a significant effect on L*, a* and chroma ($P<0.05$). Frozen samples were darker, redder and more intense in colour than chilled samples. The rate of chill had a significant effect on b* value, hue and intensity of colour (chroma). Meat cooled quickly (VFC and FF) was more blue, less yellow, hence redder in hue and less intense (chroma) in colour than samples cooled slowly (CC and CF). There was no interaction between phase and rate of cooling on colour ($P>0.05$).

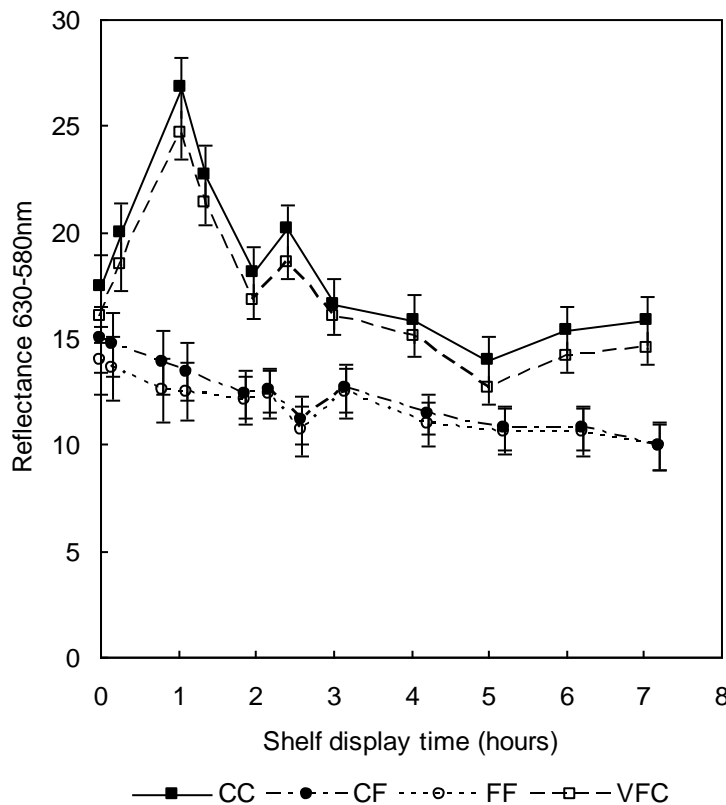


Figure 4 Change in colour from red to brown (Reflectance 630-580nm) during display period (values are means, bars = SEM, $P < 0.05$, higher values = red and lower values brown)

There was a significant effect of display time ($P < 0.001$), phase ($P = 0.046$), rate of cooling ($P = 0.010$) and an interaction between phase and display time ($P < 0.001$) for red brown colour represented by the difference in reflectance of light at 580 nm and 630 nm (Figure 4). As could be expected samples from all treatments became browner during display. Frozen samples were browner than chilled samples.

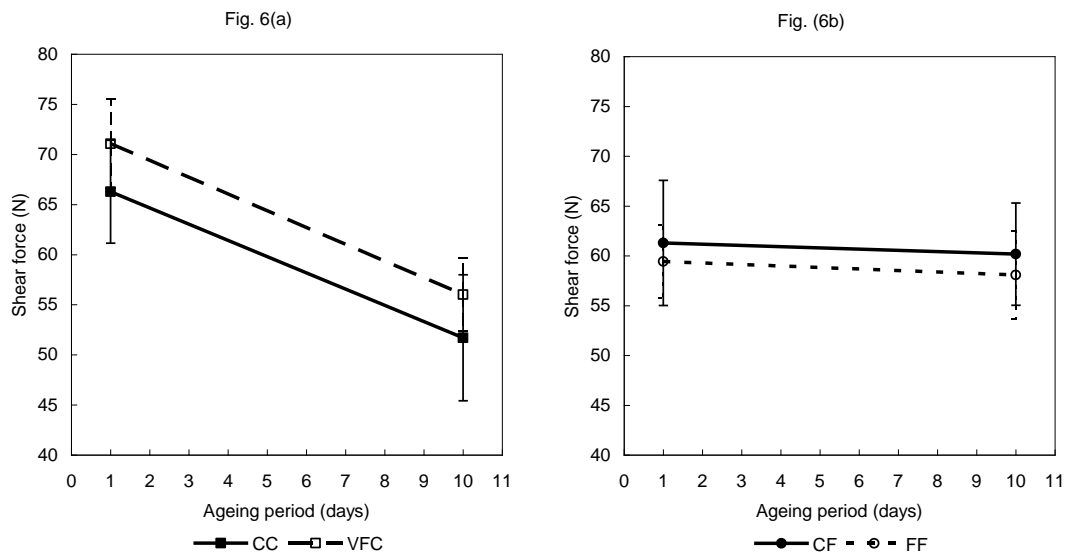


Figure 5 Shear force (N) at day 1 and day 10 of ageing for chilled (6a- CC & VFC) and frozen loins (6b- CF & FF)

There was a significant interaction ($P < 0.05$) between phase endpoint and ageing period and no effect of rate of chilling on shear force (Figure 5). Loins that were chilled had lower shear force values on day 10 compared to day 1 and for the loins that were frozen there was no difference in shear force between days 1 and 10. Loins that were frozen were lower in shear force than loins that were chilled on day 1 and not on day 10.

Table 5 Bag, drip and cooking losses for chilled loins

Parameter	Slow	Fast	LSD	P value
Bag loss	0.28	0.19	0.06	0.013
Drip loss during ageing	7.15	9.39	1.54	0.005
Cook loss day 1	24.23	23.76	1.74	0.53
Cook loss day 10	23.61	23.8	1.76	0.80

There was an effect of chilling rate on bag loss and drip loss but not cook loss in chilled loins (Table 5). Fast chilled loins lost less weight in the bag and more weight during ageing than slow chilled loins.

Table 6 Bag, drip and cooking losses for frozen loins

Parameter	Slow	Fast	LSD	P value
Bag loss	1.26	0.80	0.56	0.09
Drip loss during ageing	5.37	6.37	2.30	0.33
Cook loss day 1	25.67	23.97	2.94	0.21
Cook loss day 10	21.51	21.79	2.94	0.82

There was an effect of chilling rate ($P=0.09$) on bag loss (Table 6) but not on drip or cook loss in frozen loins ($P>0.05$).

Discussion

Effect of treatment on meat quality

Sarcomere shortening was absent from all treatments so only partly explains the relatively low shear force values on day 1 (White *et al.* 2006) for frozen samples. Being common to all treatments suggests that sarcomere shortening was prevented by events that occurred before cooling. Electrical stimulation, low pH after stimulation, restraint afforded by the vacuum packaging material and other factors associated with the type of animal may have contributed to this effect (Devine 1994). There was no correlation between pH₀ or pH_u and sarcomere length, and pH₀ had no influence on the effect of treatment on shear force when included as a covariate. However this does not rule out electrical stimulation being an important factor because there were no shortened loins to provide a comparison.

An interesting aspect of the shear force data was the reduction between days 1 and 10 that occurred for chilled product but not frozen product. This interaction between ageing time and treatments was reminiscent of “accelerated tenderisation” found with lamb loins in previous VFC experiments (Jacob *et al.* 2008). In the latter scenario the shear force was low at day 1 and did not change further with ageing to day 5. Whilst the temperature decline for the CF treatment was slower than for the FF treatment, 0°C was reached at 6h which is close to the classical definition of 0°C in 5h for VFC (Troy and Joseph 2001). This might explain why shear force values were similar in CF and FF treatments, if timing of the phase change was important.

Changes occurred in frozen samples before chilled samples for colour as well as shear force. Chilled samples (slow and fast rates) became redder before commencing to turn brown from day 1 onwards. An extended blooming period is not an uncommon finding when colour is measured with a Hunterlab reflectometer. By contrast frozen samples (slow and fast rates) showed no blooming pattern at all and simply became browner from the beginning of the display period, resembling the chilled samples from day 3 onwards.

There was no corroborative evidence to provide a reason for the difference in shear force between chilled and frozen treatments and samples were not taken to investigate proteolysis. Nevertheless these findings add to suspicions that the process of “accelerated tenderisation” is a different sequence of events to those that prevent sarcomere shortening.

VFC may entail both effects occurring together when completely effective and just one or even none when incompletely effective. In terms of potential mechanisms there are several possibilities. In the absence of electrical stimulation, prevention of sarcomere shortening remains important and VFC does this by increasing the rate of pH decline and providing physical restraint when temperatures are very low pre-rigor. In this experiment “accelerated tenderisation” was not a function of the VFC treatment and was associated with phase change rather than a faster rate of cooling to the point of phase change. Mechanisms for this “accelerated tenderisation” effect therefore require further elucidation but could include physical and or chemical factors such as non enzymatic protein degradation associated with calcium release (Takahashi 1996). Indeed this might occur subsequent to rigor development in the first 24h post-mortem.

Although the benchmark of subzero temperatures in 3 hours was reached in this experiment, there were other indications besides the shear force results to suggest that VFC was not entirely successful. The rate of pH decline was expected to be faster with VFC due to the “freeze concentration” effect accelerating glycolysis by increasing substrate concentration (Dransfield 1998). So pH_{24} should have been lower for VFC than CC and not higher as found. Frozen samples were not measured for pH at this time due to the sample being solid. Other techniques besides direct measurement of pH are available and could be used in future work.

Previous work (Jacob *et al.* 2008) found that VFC occurs close to the point of phase change; but phase change has never been included within the definition of VFC (Troy and Joseph 2001). In this experiment the loins were removed from the plate freezer at the commencement of phase change, indicated by a flattening of the temperature time profile. However the “accelerated tenderisation” effect may require phase change to be more advanced; maybe to the point where the latent heat period is complete and the temperature begins to fall at a rapid rate again. In fast chilled frozen loins (CF) this occurred 4-5 h after commencement of chilling. Clarification of this aspect may improve the understanding of the mechanism behind VFC and the specific cooling conditions required to replicate results consistently.

Treatment had a small effect on water holding capacity but probably not enough to be considered an advantage. The increase in drip loss in chilled samples may have been a function of tenderisation due to ageing (Rosenvold *et al.* 2008). As with the effect on sarcomere length, water holding capacity may have been determined prior to commencement of cooling. There was no difference between treatments in the

time taken to reach the temperature critical for protein denaturation of 35°C. Trying the faster cooling rates without electrical stimulation on the slaughter floor could be a way of testing such a hypothesis.

Practical considerations

The shear force values for slow chilled samples (CC) were relatively low and similar to those found for the control in Experiment 2; 56 and 43N at days 2 and 10 respectively. Together these results suggest that the current chilling regime at HW Greenham & sons produces an acceptable product for “budget beef” from a tenderness point of view particularly given the types of animals used. Prevention of sarcomere shortening with hot boning appears to be a component of this and may be due to a number of factors as mentioned previously.

Increasing the chilling rate had no benefit for shear force in either chilled or frozen product. Unless the apparent effect of phase change can be incorporated into the VFC treatment, there would be no benefit likely of increasing chilling rate for tenderness at this plant under current processing conditions. VFC may have an advantage if it allowed prevention of sarcomere shortening to be assured without electrical stimulation. The aim would be to reduce drip loss changes associated with electrical stimulation but further testing would be required to prove this.

Throughput of a plate freezer could theoretically be increased by using the lower carton height of 60mm used in this experiment. Chilled product reached the minimum temperature in 3 hours and frozen product within 6 hours which is considerably shorter than the conventional period of 24h. So whilst the 60mm boxes contained half the number of loins as conventional boxes the time required for processing was less than half. Practical ramifications such as labour requirements for loading and carton design would need to be considered as well.

In practice, achieving a very fast chilling regime with a plate freezer is likely to be difficult if the need to minimise the time between slaughter and commencement of chilling is important. In this experiment this time period was kept to a minimum by loading just one carton of 4 loins into the plate freezer per run. Even under these conditions the time required to reach subzero temperatures was greater than 1 hour, the time considered optimal, although inside the maximum time of 3hours.

Much greater variability in times from slaughter to commencement of chill could be expected if the plate freezer was loaded to capacity and some of the load could be expected to exceed the maximum time allowable for VFC. However this issue may

not be a consideration if phase change was in fact the critical factor rather than the time to reach minimum temperature.

Although dark, the meat was stable in colour during display including the frozen samples which failed to bloom. This may have been due to the type of cattle being dairy animals. Raines *et al* (2009) found that *m. semimembranosus* (topside) from dairy type animals was more stable in colour than that from beef animals and speculated there could be several reasons for this.

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