Meat quality of bison (*Bison bison bison*) longissimus thoracis et lumborum following very fast chilling

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Janz, J. A. M., Aalhus, J. L. and Price, M. A. 2002. **Meat quality of bison (*Bison bison bison*) longissimus thoracis et lumborum following very fast chilling**. Can. J. Anim. Sci. **82**: 327–337. Very fast chilling (VFC; internal muscle temperature of –1°C by 5 h postmortem) was achieved in the longissimus lumborum (LL), but not in the semimembranosus, of lean bison carcasses after only 4 or 6 h of chilling at –35°C. Rigorous chilling caused a shift in moisture loss from carcass cooler shrink to retail drip loss. Sides exposed to VFC conditions had darker LL colour at 24 h postmortem; however, the difference did not persist to 6 d. While chilling for 2 h at –35°C resulted in an increased shear over conventionally chilled samples, the application of VFC for 4 and 6 h decreased mean shear values and resulted in a slight improvement in tenderness consistency. Sensory evaluation panellists noted marginal, non-significant differences. Factors affecting tenderization were the physical prevention of sarcomere shortening due to surface freezing with increased chilling intensity and a contribution from proteolytic enzyme systems over time postmortem. Very fast chilling is an effective means of reducing carcass chilling time while improving tenderness in the LL of lean bison carcasses.

Key words: Bison, meat quality, very fast chilling

Janz, J. A. M., Aalhus, J. L. et Price, M. A. 2002. **Qualité du longissimus thoracis et du longissimus lumborum du bison (*Bison bison bison*) après surgélation**. Can. J. Anim. Sci. **82**: 327–337. On a réussi à surgeler (température interne du muscle de –1 °C cinq heures après l'abattage) le longissimus lumborum (LL) des carcasses de bisons à viande maigre après 4 à 6 h de refroidissement à –35 °C, mais pas le semimembranosus. Une réfrigération sévère freine la perte d'eau des carcasses de la freinte normalement observée en salle frigorifique au simple ressuage noté chez le détaillant. Les flancs surgelés présentaient un LL de couleur plus foncée 24 h après l'abattage, mais cette variation avait disparu au bout de 6 jours. Refroidir la carcasse à –35 °C pendant 2 h rend la viande moins résistante au cisaillement, comparativement aux échantillons simplement réfrigérés. En outre, 4 à 6 h de surgélation réduisent l'effort tranchant moyen, ce qui donne une viande légèrement plus tendre. Les membres du jury organoleptique ont relevé des différences marginales, quoique non significatives. Les facteurs affectant l'attendrissement comprennent la prévention physique du rétrécissement des sarcomères en raison de la congélation du muscle en surface à cause du froid plus intense et l'intervention des enzymes protéolytiques pendant la période post-mortem. La surgélation est une méthode efficace pour réduire la durée de la réfrigération et améliore la tendreté du LL dans les carcasses de bison à viande maigre.

Mots clés: Bison, qualité de la viande, surgélation

With any type of meat-producing animal, there is a need to be able to produce meat of consistent eating quality, particularly with uniform tenderness. Since bison is a relatively "new" meat choice, garnering and maintaining consumer satisfaction is essential to establishing a strong standing in the retail marketplace. Many carcass treatments are available to manipulate and maximize eating quality, and an optimum combination would decrease processing time without compromising eating quality. Blast chilling can reduce cooler time, but its use as a single treatment can result in toughening (Aalhus et al. 1991, 1994). Janz et al. (2001) showed that blast chilling (–20°C, 3 m s^{-1} air velocity, 2 h) of feedlot finished bison carcasses resulted in an increased shear force unless low voltage electrical stimulation was previously applied to temper the extreme chilling conditions. There is mounting evidence in the literature, however, that chilling at a more rapid rate than achieved with blast chilling can have a tenderizing effect.

Joseph (1996) outlined the European Union concerted action on very fast chilling (VFC) of beef. The working definition for the treatment is the attainment of an internal muscle temperature of –1°C within 5 h of stunning. There is evidence that VFC results in tender meat (Davey and Garnett 1980; Bowling et al. 1987; Sheridan 1990; Jaime et al. 1992; Sheridan et al. 1998; Aalhus et al. 2002) and determining the required time/temperature conditions and the precise mechanism of tenderization are the objectives of current and ongoing investigation. Since the VFC process is still under examination, no precise chilling guidelines exist, and VFC parameters for bison are unknown. Furthermore,

Abbreviations: **CONV**, conventional chilling; **LL**, longissimus lumborum; **LRC**, Lacombe Research Centre; **LT**, longissimus thoracis; **LTL**, longissimus lumborum et thoracis; **pH_u**, ultimate pH; **VFC**, very fast chilling

when whole sides are subject to VFC conditions, a non-uniform chilling rate amongst muscles is produced (Joseph 1996), with the temperature decline in some, but not all, attaining VFC requirements.

The objectives of the present study were to determine the time/temperature conditions required to achieve VFC in the longissimus thoracis et lumborum (LTL) of lean bison carcasses and to determine the effects on meat quality. The effects of VFC on muscle fibre morphology and postmortem biochemistry were examined to assist in the understanding of the mechanism of tenderization associated with VFC.

MATERIALS AND METHODS

Carcass Treatment

On the day prior to each of five kill dates, six to nine bison bulls (*N* total = 37), procured from two commercial feeding operations in Alberta, were transported to the lairage area of the abattoir at the Agriculture and Agri-Food Canada Lacombe Research Centre (LRC), and provided with straw bedding and water. On the morning of slaughter, animals were weighed, stunned, and processed according to typical commercial procedure with the exception of various treatments and sampling techniques as described below. By 1 h postmortem, carcasses were split, weighed, and rinsed, and paired sides were place into the chilling treatments, alternating between left and right sides within a treatment. Under conventional chilling (CONV) sides were chilling at 2°C until 24 h postmortem, whereas very fast chilling (VFC) consisted of chilling at –35°C for 2, 4, or 6 h, then at 2°C until 24 h postmortem. On the morning following slaughter, all sides were reweighed for shrink loss determination and prepared for grading by cutting through the muscle and vertebrae between the 11th and 12th ribs. Following 20 min of exposure to atmospheric oxygen, the colour of the lean tissue was objectively evaluated (ChromaMeter II, Minolta Camera Company, Japan; light source C, 2° observer angle) at three locations across the exposed longissimus surface with care to avoid areas of clearly visible connective and adipose tissues. Experienced LRC staff provided a detailed carcass evaluation including grade assignment according to current Canadian bison carcass grading standards (Canadian Food Inspection Agency 1995). From all sides, approximately 20 cm of the LTL was removed both anterior (LT) and posterior (LL) to the grade site for further analysis.

Meat Quality Assessment

At fixed intervals during carcass dressing and chilling, pH and temperature in the 12th/13th rib region of the LL were measured using an Accumet 1002 pH meter with temperature probe (Fisher Scientific, Edmonton, AB) and Orion Ingold Electrode (Udorf, Switzerland). Small (~100 g) muscle samples from the same area were also removed with a stainless steel corer. Approximately 40 g of fresh tissue was immediately prepared for cathepsin and calpastatin assay while the remainder of the sample was flash frozen in liquid nitrogen and stored at –80°C for later assay of glycogen and lactate. The first measurement was taken through the hide during exsanguination. Subsequent pH and temperature measurements were made at 1, 3, 5, 7, 9, 11, and 24 h postmortem and at a tissue depth of approximately 2.5 cm. Additional core samples were removed at 1 h postmortem, immediately following VFC treatment, and at 9 and 24 h postmortem. Enzyme assays were completed on samples collected at 0, 1 and 24 h, and 6 and 9 d postmortem.

In order to plot continuous temperature changes, portable SAPAC Temprecord data loggers (Argus Distributors, Ltd, Auckland, New Zealand) were inserted into the LL prior to the entry of sides into the coolers, and when VFC sides were removed from the –35°C conditions. A second probe was placed into the semimembranosus muscle, 9 cm above the dorsal end of the aitch bone, and retracted 3 cm from the femur, to record temperature changes in the deep hip region.

At 24 h, small (~50 g) LL samples were removed from near the 13th rib and immediately prepared for sarcomere length measurement according to methods reported by Aalhus et al. (1999). Under 1000× oil immersion phase contrast magnification (Zeiss Axioskop, West Germany), images of 10 different myofibres from each sample were captured using Image Pro Plus (Version 3.0, Media Cybernetics, Silver Springs, MD). Sarcomere lengths were measured using the Fast Fourier Transformation function that analyzed the repeating striated pattern.

Once LL and LT sections were removed from all sides at 24 h, one steak (2.54 cm thickness) was removed from the LL and immediately prepared for cooking and shear force measurement. The remaining LL and LT sections were vacuum packaged whole and stored under refrigeration at 4°C until 6 d postmortem.

Longissimus thoracis sections were used exclusively for sensory analysis, and at 6 d postmortem were divided into steaks (2.54 cm), vacuum packaged, and aged to 13 d postmortem. Sensory samples were then frozen and stored (–25°C) until analysis could be completed. From each LL section, four steaks (2.54 cm) were removed. After ultimate pH (pH_u) was recorded and a final objective colour measurement was taken from the first steak, it was weighed, placed on a styrofoam tray lined with a Dri-loc pad, overwrapped with oxygen-permeable film, and refrigerated at 4°C until 10 d postmortem, at which time drip loss was determined gravimetrically. The remaining three steaks were designated for cooking and shearing. One was prepared immediately, while the other two were weighed, vacuum packaged, and aged to 13 d postmortem. At 13 d, one steak was immediately cooked and the other was frozen for later cooking and shearing.

In preparation for cooking, steaks were weighed and a stainless steel temperature probe connected to a Hewlett Packard 34970A Data Acquisition Switch Unit was inserted into the geometric centre of each in order to continually monitor internal temperature using Hewlett Packard Benchlink Software. Steaks were placed on a preheated (200°C) Garland ED–30B electric grill (Garland Commercial Ranges, Ltd., Mississauga, ON), cooked to an internal temperature of 40°C, turned, and cooked to a final internal temperature of 72°C. When the final cooking temperature was reached, steaks were removed from the grill,

temperature probes were removed, and each steak was placed in an individual zipper lock bag. Cooked samples were immediately cooled in an ice bath to arrest cooking, then refrigerated overnight. The following morning, steaks were reweighed for cooking loss determination and four to six 19-mm- diameter cylindrical cores were removed from each steak with a stainless steel corer parallel to the grain of the meat to allow shearing perpendicular to muscle fibres. Cores were sheared using a Warner-Bratzler shear cell attached to an Instron Model 4301 Materials Testing System (Burlington, ON) with a crosshead speed of 200 mm min^{-1}. Peak shear force was recorded in kilograms and converted to N cm^{-2}.

Cathepsin and Calpastatin Assays

Immediately following collection, samples were taken to a cold room (4°C) for homogenization and centrifugation according to Goll et al. (1974). Supernatants were stored at 4°C until assayed for cathepsins B and L activity using fluorimetric assay procedures established by Barrett (1980). Soluble protein concentration of supernatants was measured using the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL).

Calpastatin was extracted from muscle samples using the modified method of Shackelford et al. (1994). Immediately following sample collection, 10 g of sample was scalpel minced at 4°C and placed in 30 mL of buffer solution before being homogenized using a Polytron PT3100 homogenizer (Brinkmann Instruments Inc., Mississauga, ON), and centrifuged at 35 000 × *g* for 1 h at 4°C. Supernatants were filtered through cheesecloth and glass wool before heating in a pre-warmed 95°C water-bath for 15 min to inactivate calpains. Coagulated protein was scrambled with a glass rod to facilitate separation of supernatant and pellet. Samples were cooled in an ice bath and centrifuged at 35 000 × *g* for 30 min at 4°C. The final supernatant volume was recorded and pH was adjusted to 7.5. Calpastatin activity was determined using the modified method of Koohmaraie (1990). Supernatant dilutions were assayed with m-calpain in the presence of either EDTA or calcium chloride in an assay medium. Bison m-calpain was previously purified from lung tissue collected during commercial slaughter. After incubation for 1 h in a 25°C water-bath, reactions were stopped with the addition of 5% trichloroacetic acid. Samples were centrifuged at 3000 × *g* for 10 min at 25°C and read on a spectrophotometer (Ultraspec 3000, Pharmacia Biotech, England) at absorbance of 278 nm.

Glycogen and Lactate Assay

Frozen samples were prepared following methods previously described by Dalrymple and Hamm (1973) and Yambayamba et al. (1996) with the exception that lactate content was read using a YSI 2300 Stat Plus glucose/lactate analyzer (YSI Incorporated, Yellow Springs, Ohio) and expressed as μmol g^{-1} fresh tissue.

Sensory Evaluation

Six experienced panelists (American Meat Science Association 1995; Jeremiah et al. 1997) were present for each of the sessions, which were held in well-ventilated, partitioned booths under 1076 lx of incandescent and fluorescent light. Distilled water and unsalted crackers were provided to cleanse the palate between samples. Stored steaks were removed from the freezer and allowed to thaw at 4°C for 24 h. Cooking was similar to that used to prepare samples for shearing. A 5-min cooling period was allowed after cooking and from each steak, six 1.3-cm^3 subsamples were cut, avoiding obvious fat and connective tissue. Samples were colour coded and placed in jars in a 70°C water bath to allow for sample temperature equilibration until provided to panelists. Each subsample was evaluated for initial and overall tenderness, juiciness, flavour desirability and intensity, amount of perceptible connective tissue, and overall palatability using 9-point scales (9 = extremely tender, extremely juicy, extremely desirable, extremely intense, none, extremely desirable; 1 = extremely tough, extremely dry, extremely undesirable, extremely bland, abundant, extremely undesirable).

Statistical Analysis

Prior to analysis, CIE L*, a*, and b* (Commision Internationale de l'Éclairage 1978) values were converted to colour (hue = arctan[b*/a*]) and colour saturation (chroma = $[a^{*2}+b^{*2}]^{0.5}$) and averaged. Carcass and meat quality data were analyzed according to an incomplete block design with animal as the blocking factor and four levels of chilling as the treatment, using the GLM procedure of SAS (SAS Institute, Inc. 1990). A Tukey-Kramer adjustment factor was applied in order to maintain a 0.05 significance level while making multiple pairwise comparisons.

In order to determine the effect of ageing time on tenderness, time was included in the model for the analysis of shear values. To further assess shifts in tenderness over the ageing period and within chilling treatments, the proportion of samples within predetermined tenderness categories was determined using the FREQ procedure of SAS with the Chi-square option. The tenderness categories, based on extensive beef quality research at the LRC (Aalhus et al. 2000), were set as follows:

Tender: <19.36 N cm^{-2}
Probably tender: 19.36 to 27.14 N cm^{-2}
Probably tough: >27.14 to 33.19 N cm^{-2}
Tough: >33.19 N cm^{-2}

The FREQ procedure was also employed to analyze for any effect of chilling treatment on grade assignment. Due to low numbers, sides rated Canada A1, A2, or A3 were combined into an A-class category for comparison to other grades.

RESULTS AND DISCUSSION

The animals procured for this study were lightweight (median 400.0 kg, range 373.0–518.5 kg) and lean with minimal subcutaneous fat cover. The resulting carcasses had little grade fat (median 1.0 mm, range 0.0–16.0 mm) and grade assignment was as follows: 1-A1, 3-A2, 1-A3, 22-B2 and 11-D1. A subjective rating of dark colour at grading resulted in B2 assignment and lack of finish in D1.

A primary objective was to establish the time/temperature conditions necessary to achieve VFC conditions (–1°C by

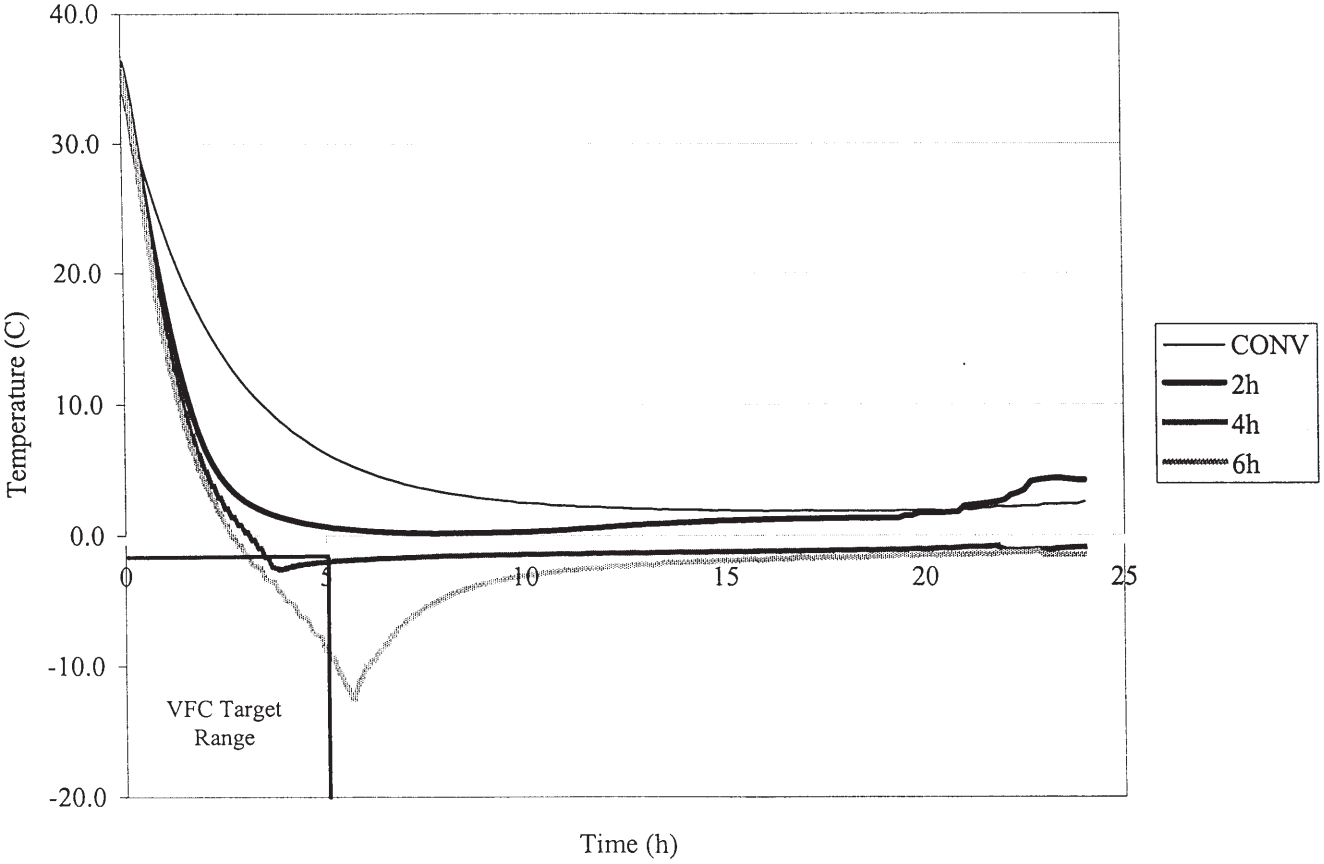

Fig. 1. Mean postmortem temperature decline of bison longissimus lumborum during conventional chilling (CONV) or chilling at –35°C for 2, 4, or 6 h (2h, 4h, 6h).

5 h postmortem) for selected bison muscles. Internal muscle temperature decline in the LL (Fig. 1) demonstrated that VFC was achieved in the loin region of sides chilled for 4 and 6 h at –35°C. Chilling for 2 h at –35°C was insufficient to achieve VFC and produced a temperature decline similar to that following blast chilling (Janz et al. 2001). The deep hip region is an area of thick muscle mass and none of the time/temperature combinations was sufficient to meet VFC conditions, with internal semimembranosus temperature near 20°C at 5 h.

Sarcomere Length

Decreased muscle temperature compromises the ability of internal muscle cell membranes to sequester calcium ions that are essential to contraction (Cornforth et al. 1980). Cold exposure of pre-rigor muscle will, therefore, result in contraction since energy sources are still available. The potential for contraction is maximized with extreme carcass chilling since membranes are cold-compromised early in the pre-rigor period. The present results for VFC treatments, however, appear counterintuitive; that is, sides exposed to the more extreme temperatures had the longest sarcomere lengths (Table 1). Davey and Garnett (1980) suggested that rapid chilling results in freezing of the outer surface of the carcass and that this frozen crust provides restraint sufficient to counter muscle contraction. In the present study, crust freezing of sides exposed to –35°C did occur and the depth to which freezing penetrated the surface increased with the length of time of cold exposure.

Shear Force

At each postmortem evaluation time, a similar pattern of mean LL shear force was present amongst treatments (Table 1). Carcass chilling for 2 h at –35°C tended to produce the toughest LL samples while the more rigorous chilling treatments, those satisfying VFC requirements, tended to generate more tender samples. Conventional chilling resulted in intermediate shear values. In general, comparatively longer sarcomere length equated to lower shear force. Where 4 and 6 h of extreme chilling was applied to the carcass, significantly longer sarcomere lengths resulted and shear force values were lower than those from the 2 h and CONV treatments. This phenomenon may have been due to the physical restraint of the frozen outer surface of the carcass counteracting the tendency for the muscles to contract during the onset of rigor mortis.

Retail consumers generally purchase a single cut of meat (i.e., one roast or steak) or cuts from a single animal

Table 1. Mean sarcomere length (μm; SEM) at 24 h postmortem and shear force (N cm^{-2}; SEM) values of bison longissimus lumborum at various times postmortem following conventional chilling (CONV) or chilling at –35°C for 2, 4, or 6 h (2h, 4h, 6h)

	Chilling treatment				
	CONV	2 h	4 h	6 h	*P*
Sarcomere length	1.34*a* (0.02)	1.48*ab* (0.05)	1.64*bc* (0.05)	1.73*c* (0.05)	<0.01
Shear force					
24 h	47.48*a* (1.12)	50.41*a* (2.43)	40.73*a* (2.55)	31.50*b* (2.55)	<0.01
6 d	37.76*a* (1.37)	41.08*a* (2.96)	28.15*b* (3.11)	24.86*bc* (3.46)	<0.01
13 d	30.29*a* (0.96)	34.44*a* (2.08)	25.86*ab* (2.19)	17.81*b* (2.30)	<0.01
13 d/Frozen	27.49*a* (0.92)	33.47*b* (2.00)	23.93*ac* (2.10)	17.39*d* (2.10)	<0.01

a–d Means in same row followed by different letters are significantly different at 0.05 level.

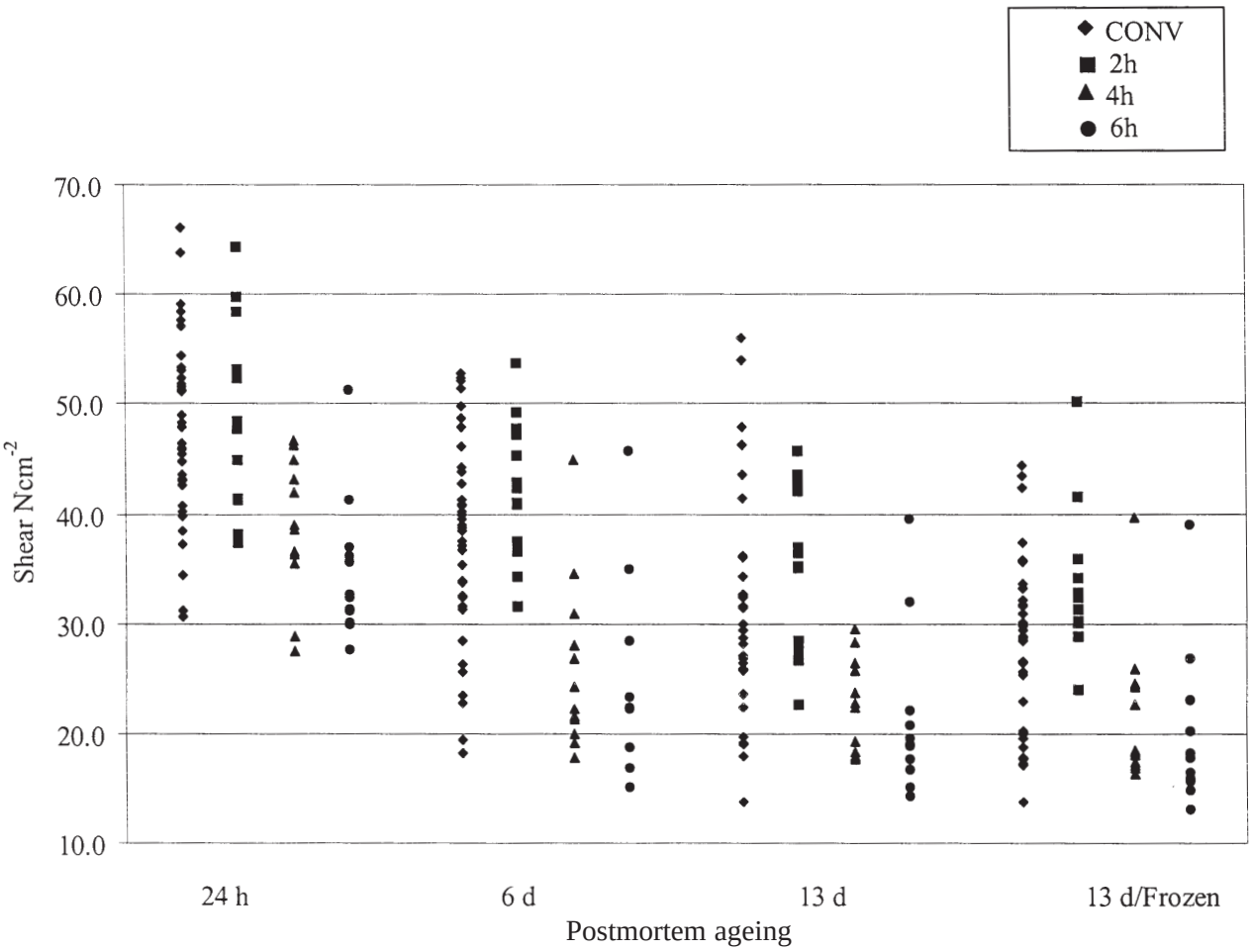

Fig. 2. Distribution of individual bison longissimus lumborum shear values within chill treatment over postmortem ageing time.

(i.e., bulk purchase of one side or quarter). As such, the need for consistency of product quality is of utmost importance to ensure a satisfied consumer who will receive comparable meat quality with subsequent purchases. While a particular carcass treatment may improve average tenderness in a given cut, as was demonstrated above, this is little guarantee that the same cut from all treated carcasses will be equally tender. When individual shear force values were plotted (Fig. 2) to demonstrate their variability, however, a slight improvement in tenderness consistency

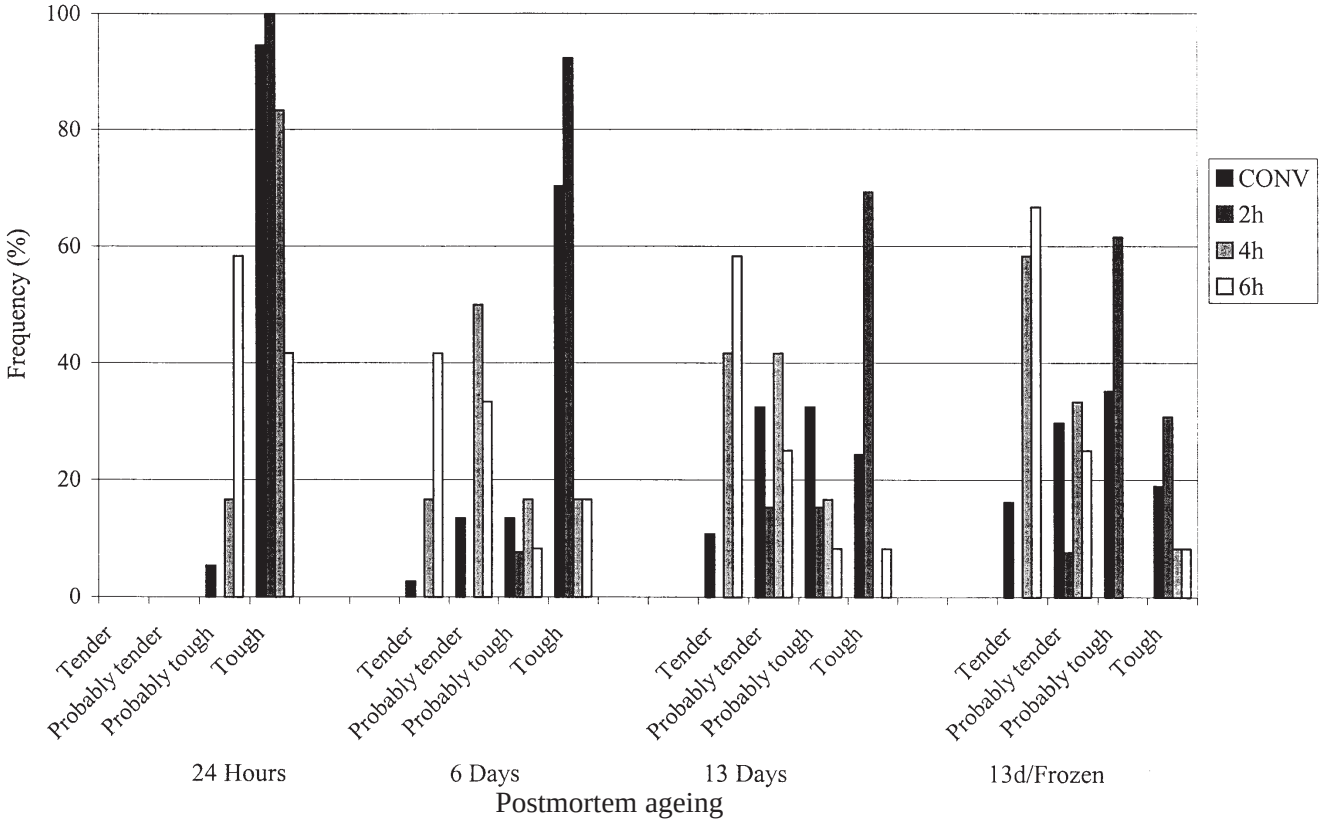

Fig. 3. Frequency distribution of bison longissimus lumborum samples from each chill treatment across various tenderness categories.

Table 2. Mean sensory panel ratings[z] (SEM) of bison longissimus thoracis steaks within chill treatment and following 13 d ageing and frozen storage

	Chilling treatment				
	CONV	2 h	4 h	6 h	*P*
Initial tenderness	4.74	5.04	4.67	4.32	0.75
	(0.19)	(0.43)	(0.43)	(0.43)	
Juiciness	5.02	5.16	5.03	5.34	0.66
	(0.11)	(0.25)	(0.25)	(0.25)	
Flavour desirability	5.53	5.41	5.49	5.72	0.77
	(0.09)	(0.20)	(0.20)	(0.20)	
Flavour intensity	5.73	5.90	5.59	5.98	0.20
	(0.06)	(0.14)	(0.14)	(0.14)	
Amount perceptible connective tissue	6.24	6.18	6.01	5.78	0.54
	(0.14)	(0.32)	(0.32)	(0.32)	
Overall tenderness	4.93	5.01	4.86	4.43	0.70
	(0.17)	(0.39)	(0.39)	(0.39)	
Overall palatability	4.83	4.90	4.81	4.48	0.79
	(0.14)	(0.32)	(0.32)	(0.32)	

[z]9-point scale (9 = extremely tender, extremely juicy, extremely desirable, extremely intense, none, extremely desirable; 1 = extremely tough, extremely dry, extremely undesirable, extremely bland, abundant, extremely undesirable).

was evident amongst the 4 h and 6 h data. While an eating experience in the "tender" (shear <19.36 N cm^{-2}) or "probably tender" (shear 19.36– 27.14 N cm^{-2}) category could not be guaranteed, it was more likely following these treatments.

As expected, the effect of postmortem ageing was highly significant ($P < 0.01$) with a general decrease in shear as ageing time increased. Figure 3 represents the shifting of samples amongst tenderness categories within chilling treatment and across the ageing period. It is interesting to note that samples from the 2 h at –35°C never entered the "tender" (<19.36 N cm^{-2}) category. There was a moderate shift towards "tender" within the CONV group, but the treatments that invoked VFC conditions represented the greatest proportion of "tender" and "probably tender" (>19.36–<27.14 N cm^{-2}) samples.

Table 3. Mean cooler shrink, drip loss, package loss, and cooking loss values (mg g^{-1}; SEM) of bison longissimus lumborum following conventional chilling (CONV) or chilling at –35°C for 2, 4, or 6 h (2h, 4h, 6h)

	Chilling treatment				
	CONV	2 h	4 h	6 h	*P*
Cooler shrink	19.24*a*	7.65*b*	2.29*c*	–2.36*d*	<0.01
	(0.39)	(0.85)	(0.89)	(0.89)	
Drip loss	22.83*a*	21.72*a*	32.02*b*	40.95*c*	<0.01
	(0.96)	(2.08)	(2.19)	(2.19)	
Total shrink and drip	42.07*a*	29.37*b*	34.32*c*	38.59*ac*	<0.01
	(0.91)	(1.97)	(2.97)	(2.07)	
Package loss					
13 d	2.73*a*	2.54*a*	3.88*b*	4.09*b*	<0.01
	(0.12)	(0.26)	(0.27)	(0.27)	
13 d/frozen	6.89	6.40	6.82	6.25	0.29
	(0.17)	(0.36)	(0.38)	(0.38)	
Cooking loss					
24 h	21.12	21.24	20.32	19.06	0.50
	(0.57)	(1.23)	(1.31)	(1.31)	
6 d	23.28	23.65	22.59	23.94	0.92
	(0.58)	(1.26)	(1.33)	(1.33)	
13 d	22.99	21.77	24.59	21.06	0.34
	(0.53)	(1.15)	(1.21)	(1.21)	
13d/frozen	27.81	26.88	26.73	28.77	0.65
	(0.55)	(1.17)	(1.26)	(1.23)	

a–d Means in same row followed by different letters are significantly different at 0.05 level.

Sensory Evaluation

Taste panelists noted only marginal differences amongst samples within each evaluation category, and statistical analysis showed that there was no significant difference amongst treatment groups (Table 2). While no one treatment resulted in a marked improvement in any evaluation category, there was no clearly detectable detrimental effect of any particular treatment on LT steaks. It is possible that the 13 d ageing period followed by frozen storage minimized differences amongst treatments; however, there were significant treatment differences amongst treatments following instrumental evaluation (Table 1) of LL steaks following similar ageing and storage.

Cooler Shrink, Drip Loss, and Package Loss

Rigorous carcass chilling significantly decreased, and prevented in the case of the 6 h treatment, evaporative cooler shrink loss (Table 3) due to the formation of a frozen outer surface crust. There was a significant effect of chilling treatment on LL drip loss (Table 3) with the more extreme VFC treatments (4 and 6 h at –35°C) resulting in greater weight loss than that from the milder chilling treatments. The extent of tissue freezing increased with chilling intensity and the subsequent increase in drip loss is likely due to more extensive tissue disruption resulting from ice crystal formation with freezing. At a point between 2 and 4 h of –35°C chilling there was an opportunity to decrease cooler shrink loss from lean bison sides and reap the VFC tenderness benefit without causing an excessive amount of retail drip loss. When cooler shrink and drip loss were combined, the greatest total moisture loss occurred with the CONV chilling treatment followed by the 6 h, 4 h, and 2 h treatments, respectively (Table 3).

There was a significant effect of carcass chilling treatment on package loss (Table 3), the weight of fluid remaining in the packaging material following 13 d of refrigerated storage. With a trend similar to that observed with drip loss, the more extreme chilling treatments resulted in a slightly greater weight loss, again due to tissue damage and moisture loss with freezing and thawing. Amongst samples that were aged and then stored in a frozen state, there was no effect of chilling treatment on package loss values and all samples displayed a sizeable weight loss. In this case, all samples were frozen and sustained a similar degree of freezing damage regardless of previous carcass chilling conditions.

Cooking Loss

No significant carcass chilling effect on cooking loss from LL steaks was observed at any cooking time (Table 3). Cooking loss represents the loss of moisture, fat, and volatile components of meat during the cooking process. The lack of treatment effect on this variable may be due to the fact that the use of extreme heat was sufficient to "force" weight loss, with all samples responding similarly regardless of previous carcass chilling treatment.

Indicators of Postmortem Metabolism

Carcass chilling treatment did not have a significant effect on the postmortem intramuscular concentrations of glycogen and lactate in the LL (Table 4) with the exception of the 24 h lactate concentration when the CONV and 2 h treatments resulted in greater lactate concentration ($P < 0.01$)

Table 4. Mean glycogen and lactate concentration (μmol g^{-1}; SEM) and pH values (SEM) of bison longissimus lumborum at various times during conventional carcass chilling (CONV) or chilling at –35°C for 2, 4, or 6 h (2h, 4h, 6h)

	Chilling treatment				
	CONV	2 h	4 h	6 h	*P*
Glycogen					
0 h	92.72[z]	–	–	–	–
	(4.26)				
1 h	90.42	89.22	90.59	89.62	0.95
	(1.00)	(2.18)	(2.28)	(2.28)	
Post-chilling[y]	75.90	78.26	79.20	77.12	0.67
	(1.89)	(3.51)	(3.69)	(8.07)	
9 h	71.62	71.29	67.61	69.43	0.80
	(1.53)	(2.84)	(2.99)	(5.62)	
24 h	53.45	55.03	55.68	54.54	0.80
	(1.20)	(2.59)	(2.72)	(2.72)	
Lactate					
0 h	14.61[z]	–	–	–	–
	(1.84)				
1 h	24.72	24.41	24.67	25.40	0.96
	(0.55)	(1.19)	(1.25)	(1.25)	
Post-chilling[y]	48.86	43.29	43.75	47.25	0.73
	(2.19)	(4.07)	(4.07)	(9.36)	
9 h	58.18	59.58	61.32	54.67	0.47
	(1.61)	(2.99)	(3.14)	(5.90)	
24 h	79.40*a*	78.61*a*	74.10*b*	72.94*b*	<0.01
	(0.75)	(1.63)	(1.71)	(1.71)	
pH					
0 h	6.99[z]	–	–	–	–
	(<0.01)				
1 h	6.75	6.73	6.72	6.68	0.09
	(0.01)	(0.02)	(0.02)	(0.02)	
3 h	6.48	6.52	NA[x]	NA[x]	0.47
	(0.02)	(0.05)			
5 h	6.29	6.32	6.44	NA[x]	0.53
	(0.03)	(0.07)	(0.13)		
7 h	6.40	6.28	6.42	NA[x]	0.36
	(0.03)	(0.07)	(0.15)		
9 h	6.33	6.15	6.40	NA[v]	0.06
	(0.03)	(0.07)	(0.08)		
11 h	6.19	6.08	6.22	6.23	0.34
	(0.02)	(0.05)	(0.06)	(0.14)	
24 h	5.77*a*	5.78*a*	5.85*ab*	5.92*b*	<0.01
	(0.01)	(0.02)	(0.03)	(0.03)	
6 d	5.72	5.73	5.72	5.70	0.69
	(0.01)	(0.02)	(0.02)	(0.02)	

[z]One sample collected at 0 time to represent the carcass.
[y]Immediately after completion of VFC treatment.
[x]With sides still in –35°C cooler, pH data not collected.
[v]Sides frozen too firmly for probe insertion and pH measurement.
a, *b* Means in same row followed by different letters are significantly different at 0.05 level.

than the 4 h and 6 h VFC treatments. This trend paralleled LL pH decline in which there was no significant difference amongst chilling treatments until 24 h postmortem. At this time, the pH resulting from 6 h VFC was significantly greater than the milder chilling treatments (Table 4).

While the mean values for each chill treatment were within the normal pH_u range (5.4–5.8), there were samples from all treatments with high pH_u (>5.9), which was reflected in the subjective colour evaluation at grading when these carcasses were rated "dark", "very dark", or "black". Since this was not an occurrence unique to any particular chilling treatment, there were additional factors (e.g., antemortem stress) at play in the development of high pH_u.

Colour

Since chilling rate may influence the rate of postmortem metabolism and pH decline, the temperature to which carcasses are exposed during cooling can be a potent influence on colour development (Renerre 1990). A common observation following the application of rapid chilling processes is darker meat colour (Bowling et al. 1987; Aalhus et al. 1994). In the present study, samples from the more rigorous chilling treatments (4 and 6 h at –35°C) had a lower L* (lightness) value at 24 h postmortem as compared to CONV and 2 h at –35°C (Table 5). While the colour (hue) of samples from all treatments was similar, the more rigorous chill treatments resulted in less colour intensity (lower chroma

Table 5. Mean objective colour values (SEM) of bison longissimus lumborum at 24 h and 6 d postmortem following conventional chilling (CONV) or chilling at –35°C for 2, 4, or 6 h (2h, 4h, 6h)

	Chilling treatment				
	CONV	2 h	4 h	6 h	*P*
24 h					
L*	30.09*a*	30.03*a*	27.50*b*	27.18*b*	<0.01
	(0.17)	(0.36)	(0.38)	(0.38)	
Hue	20.92	20.42	20.09	20.64	0.11
	(0.16)	(0.35)	(0.37)	(0.37)	
Chroma	19.47*a*	18.35*ab*	16.84*b*	16.99*b*	<0.01
	(0.23)	(0.50)	(0.52)	(0.52)	
6 d					
L*	32.77	32.24	32.82	33.26	0.34
	(0.16)	(0.35)	(0.37)	(0.37)	
Hue	21.94	21.99	22.51	22.53	0.43
	(0.20)	(0.42)	(0.45)	(0.45)	
Chroma	22.27	21.16	22.01	23.66	0.09
	(0.28)	(0.60)	(0.63)	(0.63)	

a, *b* Means in same row followed by different letters are significantly different at 0.05 level.

Table 6. Effects of chilling treatment on soluble protein concentration (mg mL^{-1}) and cathepsin activity (nmol g^{-1} protein min^{-1}) and time postmortem on soluble protein concentration, and cathepsin and calpastatin activities (units g^{-1}) in bison longissimus lumborum

	Chilling treatment				
	CONV	2 h	4 h	6 h	*P*
Soluble protein	5.63*ab*	5.31*a*	5.98*ab*	6.06*b*	<0.01
	(0.07)	(0.15)	(0.15)	(0.16)	
Cathepsin activity	0.059	0.060	0.058	0.064	0.69
	(0.002)	(0.004)	(0.004)	(0.004)	
	Time postmortem				
	0 h	24 h	6 d	9 d	*P*
Soluble protein	5.50*a*	5.93*b*	5.81*ab*	NA	0.01
	(0.12)	(0.08)	(0.08)		
Cathepsin activity	0.056	0.063	0.061	NA	0.09
	(0.002)	(0.002)	(0.002)		
Calpastatin activity	2.91*a*	2.89*a*	1.79*b*	1.27*c*	<0.01
	(0.08)	(0.08)	(0.08)	(0.08)	

a–c Means in same row followed by different letters are significantly different at 0.05 level.

value). These colour differences were no longer present at 6 d postmortem, however, when pH_u was similar amongst treatment groups.

Chilling treatment did not significantly affect grade assignment, however, further examination of the assignment of A-class grades was warranted. Due to a general lack of finish and colour quality amongst the carcasses, only five A-class grades were assigned, all of these were awarded to CONV sides. Comparison of paired sides showed the VFC treated side was downgraded to B2 in each case due to dark colour. The colour acceptability for each A-class side, however, was "borderline"; that is, none were rated "bright" but, rather, "approaching medium dark". It appears then, that in each case VFC treatment of the paired side was enough to tip the balance of subjective colour quality away from A-class to result in a B2 grade. For all other carcasses, there was no colour discrepancy between sides.

The results indicate potential for a chilling-induced colour differential in bison carcasses of limited finish and that this may influence grade assignment. Since colour variability is transient, however, consumer preference would not be influenced by alternative chilling treatments since there is a delay between carcass grading and final product marketing.

Cathepsin Activity and Soluble Protein Concentration

Cathepsin activity per gram tissue increased ($P < 0.05$) with an increase in both chilling severity and time postmortem (Table 6) corresponding to improvements in tenderness as reported in Table 1. Hence, the increase in cathepsin activity could be thought to be a direct cause of improved tenderness. Across the same chill treatments and time period, however, the concentration of soluble protein in the samples also increased (Table 6). When cathepsin activity was expressed per unit of protein, there was no longer a significant treatment effect (Table 6) or an apparent relationship to tenderness.

In the absence of additional clarifying data, two concepts about the mechanism responsible for the discrepancy in cathepsin activity arise. The change in protein concentration may have been a result of changes in muscle moisture content. Applied treatments could have served to concentrate cell contents and, in this case, the activity of cathepsin per unit protein would have remained constant over time. Alternatively, the measured differences in soluble protein may have arisen due to a change in protein extractability. According to Lawrie (1998) the soluble protein fraction has a greater extractability, and therefore a higher concentration, with a higher muscle pH. With a more rapid pH decline, sarcoplasmic proteins precipitate to form insoluble aggregates with the myofibrillar fraction (Bendall and Wismer-Pedersen 1962). As well, lower sample temperature is associated with greater protein extractability (Wierbicki et al. 1956). In the present work, as chilling intensity increased, the delay of postmortem pH decline was inhibited while chilling was accelerated (Table 4 and Fig. 1).

Because of the complexity of protein and cathepsin extraction at different sampling times (variation in pH, temperature, moisture content), the authors cannot say with certainty that the treatments were the cause of variations in cathepsin activity or whether cathepsin activity was responsible for changes in tenderness.

Calpastatin Activity

The calpain system is composed, primarily, of two proteolytic enzymes (μ- and m-calpain) and their specific inhibitor (calpastatin) (Shackelford et al. 1994). Calpain activation requires the presence of calcium, freely available in the sarcoplasm of postmortem myofibres. There is evidence that in postmortem muscle tissue only μ-calpain contributes to tenderization since the levels of free calcium present within the cells is below the concentration required to activate m-calpain (Koohmaraie et al. 1987). Similar to the work of Shackelford et al. (1994), only the activity of calpastatin was measured in this experiment and can, to a limited extent, be used as an indicator of the state of the system.

While the applied carcass chilling treatments did not affect calpastatin activity in LL samples, time postmortem did play a significant role (Table 6). After 24 h postmortem, activity of calpastatin declined steadily suggesting that the contribution of the calpain system may increase with ageing time. Koohmaraie et al. (1987) suggested that the decline in calpastatin activity was a result of its degradation once μ-calpain was activated. Because of its autolytic nature, the system cannot function indefinitely, eventually degrading itself with a subsequent loss of proteolytic activity (Koohmaraie 1992).

CONCLUSIONS

Very fast chilling conditions were successfully induced in the longissimus muscle of lean bison carcasses by applying a cooler temperature of –35°C for 4 or 6 h. Chilling for 2 h at –35°C was not sufficient to reach VFC and merely created blast chilling conditions. None of the treatments generated VFC in the semimembranosus muscle. As compared to CONV, extreme carcass chilling resulted in darker LL colour at 24 h postmortem and this affected grade assignment in several carcasses, however, no colour differential remained by 6 d postmortem. Very fast chilling decreased or prevented cooler shrink and increased drip loss over CONV. Total weight lost due to shrink and drip was greatest following CONV, while VFC resulted in a shift in moisture loss from the cooler to the retail package.

The application of VFC for 4 and 6 h improved mean shear values while 2 h at –35°C increased shear over CONV samples, however, none of the treatments resulted in a decreased tenderness variability. Factors that may have been responsible for tenderization were the prevention of sarcomere shortening with increased chilling intensity and a contribution from proteolytic enzyme systems over time postmortem.

Very fast chilling can be employed to rapidly chill carcasses while resulting in a tender loin muscle. The impact on colour at the time of grading, the shift in moisture loss distribution, and the impact on other muscles are factors for further consideration.

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Aalhus, J. L., Best D. R., Costello, F. and Jeremiah, L. E. 1999. A simple, on-line processing method for improving beef tenderness. Can. J. Anim. Sci. **79**: 27–34.

Aalhus, J. L., Jeremiah, L. E., Dugan, M. E. R., Larsen, I. L., Best, D. R., Thacker, R., Costello, F. and Gibson, L. 2000. Beef tenderness strategy. A final report to the Canadian Cattlemen's Association, Calgary, AB.

Aalhus, J. L., Jones, S. D. M., Lutz, S., Best, D. R. and Robertson, W. M. 1994. The efficacy of high and low voltage electrical stimulation under different chilling regimes. Can. J. Anim. Sci. **74**: 433–442.

Aalhus, J. L., Robertson, W. M., Dugan, M. E. R. and Best, D. R. 2002. Very fast chilling of beef carcasses. Can. J. Anim. Sci. **82**: 59–67.

Aalhus, J. L., Tong, A. K. W., Robertson, W. M. and Jones, S. D. M. 1991. The effect of rapid chilling on beef quality. ICMST **37**: 220–223.

American Meat Science Association 1995. Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. National Live Stock and Meat Board, Chicago, IL.

Barrett, A. J. 1980. Fluorimetric assays for cathepsin B and cathepsin H with methylcourmarylamide substrates. Biochem. J. **187**:909–912.

Bendall, J. R. and Wismer-Pedersen, J. 1962. Some properties of the fibrillar proteins of normal and watery pork muscle. J. Food Sci. **27**: 144–159.

Bowling, R. A., Dutson, T. R., Smith, G. C. and Savell, J. W. 1987. Effects of cryogenic chilling on beef carcass grade, shrinkage and palatability characteristics. Meat Sci. **21**: 67–72.

Canadian Food Inspection Agency 1995. Livestock and poultry carcass grading regulations, office consolidation. Part VI, Sections 48–54, Schedules I-III. Also [Online] Available: http://laws.justice.gc.ca/en/C-0.4/SOR–92-541/index.html [11 January 2002].

Commision Internationale de l'Éclairage. 1978. Recommendations on uniform color spaces-colour difference equations-psycometric color terms. Pages 8–12 *in* Publication No. 15, Supplement No. 2. Paris, France.

Cornforth, D. P., Pearson, A. M. and Merkel, R. A. 1980. Relationship of mitochondria and sarcoplasmic reticulum to cold shortening. Meat Sci. **4**: 103–121.

Dalrymple, R. H. and Hamm, R. 1973. A method for the extraction of glycogen and metabolites from a single muscle sample. J. Food Technol. **8**: 439–444.

Davey, C. L. and Garnett, K. J. 1980. Rapid freezing, frozen storage and the tenderness of lamb. Meat Sci. **4**: 319–322.

Goll, D. E., Young, R. B. and Stromer, M. H. 1974. Separation of subcellular organelles by differential and density gradient centrifugation. Recip. Meat Conf. **27**: 250–290.

Jaime, I., Beltrán, J. A., Ceña, P., López-Lorenzo, P. and Roncalés, P. 1992. Tenderisation of lamb meat: Effect of rapid postmortem temperature drop on muscle conditioning and aging. Meat Sci. **32**: 357–366.

Janz, J. A. M., Aalhus, J. L. and Price, M. A. 2001. Blast chilling and low voltage electrical stimulation influences on bison (*Bison bison bison*) meat quality. Meat Sci. **57**: 403–411.

Jeremiah, L. E., Gibson, L. L. and Burwash, K. L. 1997. Descriptive sensory analysis: the profiling approach. Technical Bulletin 1997–2E. Agriculture and Agri-Food Canada, Lacombe, AB.

Joseph, R. L. 1996. Very fast chilling of beef and tenderness-a report from an EU concerted action. Meat Sci. **43**: S217–S227.

Koohmaraie, M. 1990. Quantification of Ca^{2+}-dependent protease activities by hydrophobic and ion-exchange chromatography. J. Anim. Sci. **68**: 659–665.

Koohmaraie, M. 1992. Role of the neutral proteinases in postmortem muscle protein degradation and meat tenderness. Recip. Meat Conf. **45**: 63–71.

Koohmaraie, M., Seideman, S. C., Schollmeyer, J. E., Dutson, T. R. and Crouse, J. D. 1987. Effects of post-mortem storage on Ca^{++}-dependent proteases, their inhibitor and myofibril fragmentation. Meat Sci. **19**: 187–196.

Lawrie, R. A. 1998. Pages 108–109 *in* Lawrie's meat science. Woodhead Publishing Limited, Cambridge, UK.

Renerre, M. 1990. Review: factors involved in the discoloration of beef meat. Intl. J. Food Sci. Technol. **25**: 613–630.

SAS Institute, Inc. 1990. SAS user's guide. Version 6, 4th ed. SAS Institute, Inc., Cary, NC.

Shackelford, S. D., Koohmaraie, M., Cundiff, L. V., Gregory, K. E., Rohrer, G. A. and Savell, J. W. 1994. Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner-Bratzler shear force, retail yield, and growth rate. J. Anim. Sci. **72**: 857–863.

Sheridan, J. J. 1990. The ultra-rapid chilling of lamb carcasses. Meat Sci. **28**: 31–50.

Sheridan, J. J., McGeehin, B. and Butler, F. 1998. Effects of ultra-rapid chilling and electrical stimulation on the tenderness of lamb carcass muscles. J. Muscle Foods. **9**: 403–417.

Wierbicki, E., Kunkle, L. E. Cahill, V. R. and Deatherage, F. E. 1956. Post mortem changes in meat and their possible relation to tenderness together with some comparisons of meat from heifers, bulls, steers and diethylstilbestrol treated bulls and steers. Food Technol. **10**: 80–86.

Yambayamba, E. S. K., Aalhus, J. L., Price, M. A. and Jones, S. D. M. 1996. Glycogen metabolites and meat quality in feed-restricted re-fed beef heifers. Can. J. Anim. Sci. **76**: 517–52.