PROGRESS IN VERY FAST CHILLING OF BEEF: EFFECT ON TENDERNESS AND RELATED BIOCHEMICAL PARAMETERS

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INTRODUCTION

Chilling has been the common treatment for dressed carcasses in all abattoirs for decades. The established rule of Bendall (1972) to avoid meat toughening by not chilling below 10°C sooner than 10 h pm has conditioned the chilling treatment. Over the last years however, an extra microbial hazard as well as increasing evaporative weight loss are produced by a conventional chilling (Joseph 1996). Several attempts to point out the effect of rapid chilling on meat quality revealed that meat was no tough after this treatment, or was even more tender than that obtained by conventional chilling. Our research team (Beltrán et al., 1986) reported that excised lamb muscles cooled to 0°C within 4 h pm were more tender that muscles chilled to 4 °C. Bowling et al., (1987), demonstrated that beef sides chilled to -1±1°C in 5 h were more tender that control sides subjected to conventional chilling. Sheridan (1990) reported that chilling lamb carcasses to 1°C within 3 h pm gave rise to meat as tender as that of control carcasses. As a consequence of all these findings the European Union funded a concerted action to coordinate investigation on very fast chilling (VFC), in beef (Joseph 1996). VFC conditions have been defined as 0°C at the inner part in 4-5°C hours postmortem (Dransfield & Roncalés, 1998).
The aim of the present study is to corroborate biochemical evidences, alredy seen in lamb ((Jaime et al., (1992)), which could explain the tenderising effect of very fast chilling in beef.

MATERIALS AND METHODS

Experiment 1: Two bovine *M. sternomandibularis* were removed from the carcass within 1.5 hours after slaughter. One of them was very fast chilled (VFC) by storage in a -80 °C freezer during 1 h. and then in a -20 °C freezer for 1.5 h. Thus VFC sample reached an internal temperature of 0 °C within 3.5 hours after slaughter, without appreciable freezing of the surface; in them the VFC sample was kept in a cold room at 1±1 °C. The other muscle was subjected to conventional chilling (CC) in a cold room at 1±1 °C, muscle them reached an internal temperature of 0 °C within 30 h postmortem. The pH was measured by a Hanna pH-meter with a combined glass penetration electrode at 35 h pm (state of rigor). Free calcium concentration was determined by atomic absorption according to Willis (1960) at 5 h and 35 h pm. Calcium-dependent proteolytic activity was assayed according to the procedure described by Koohmaraie et al. (1986 y 1987) at 24 h pm. Sarcomere length was measured using an immersion objective (X100) and graduated ocular (X10) on a phase contrast Nikon microscope at 35 h pm. The tenderness was determinated at 14 days pm using a INSTRON mod.4301 (Warner-Bratzer shear blade).
Experiment 2: Two bovine *M. sternomandibularis* were excised from the carcass within 1 h after slaughter. They were wrapped into a non-permeable plastic bags and subjected either to VFC conditions by immersion in a glycol bath at -6 °C or to conventional chilling in a refrigerated room at 1±1 °C. Thus the VFC sample reached an internal temperature 0 °C within 2.5 h, and CC sample reached the same temperature within 30 h pm. When VFC samples reached this temperature were stored in a cold room at 1±1 °C. Identical parameters to experiment 1 were determined, the proteolytic activity of the calpain system was assayed at 2.5 h and 3 days pm. Instrumental and a sensory evaluation of tenderness were carried out at 10 days pm. Sensory tenderness was scored using a 9-point scale, 9 denoted extremely tender and 1 denoted extremely tough.
Experiment 3: Six *Longissimus thoracis et lumborum* muscles were obtained 1 h pm with the cord and ribs. Three ot them were subjected to conventional chilling in a cold room at 1±1 °C. And the other three were very fast chilled by inmersion in a container with N_2 vapor and blowing CO_2 until 0 °C was reached at the inner part of muscles (5 h pm), thereafter, the muscles were stored at ambient temperature for 6 h., and finally at 1±1 °C in a cold room. Samples VFC showed an appreciable freezing of the surface.

Value of pH was measured at 32 h pm (state of rigor mortis), sarcomere length was determined at 32 h pm and free calcium concentration was assessed at 4 h., 32 h. and 3 days pm, as in the experiment 1. Tenderness at 3 and 8 days pm was studied either with a Texture Analyser mod. TA XT2 (Warner-Bratzler shear blade) or by sensory analysis. The rate of proteolysis was determined by electrophoresis, where myofibrillar proteins were obtained as described by Olson et al. (1976). SDS-PAGE was carried out using a phast system.

RESULTS AND DISCUSSION

Experiment 1 and 2: As a result of treatment, *M. sternomandibularis* VFC (table 1) showed a higher ultimate pH, according to Jaime et al., (1992 and 1993) in lamb and Van Laack et al., (1996) in pigs. VFC meat caused an increase of calcium release to the myofibrillar space, as Jaime et al., (1992 and 1993) evidentiated in lamb. There was no difference between meat tenderness after both chilling treatments. Nevertheless, it must be emphasised that *M. sternomandibularis* possesses a high amount of connective tissue, so that maximum stress is high in both cases. Experiment 2 gave similar biochemical results than experiment 1 (table 2.1). Sensory tenderness values evidentiated a slight difference between VFC and CC samples; panel members found more tender the meat from VFC treatment (table 2.2). Even so, all samples were assessed as tough, certainly due to the high concentration on collagen in *M. sternomandibularis*.
Experiment 3: Biochemical parameters of *Longissimus thoracis et lumborum* showed no differences with results of experiment 1 and 2 (table 3) Calpain activation brought about tender meat (figure 1) assessed either by instrumental or sensory techniques. This was in agreement with Bowling et al., 1987 and O'Mahoney et al. 1997 in beef, and Beltrán et al., 1986 and Jaime et al., 1992 in lamb. Electrophoretic study showed an increased rate of proteolysis in VFC sample (figure 2).These results are coincident with those obtained by Demeyer et al., (1996) in beef and Beltrán et al., (1986) and Jaime et al. (1992) in lamb. VFC treatment caused the freezing of the surface of muscle; this fact avoided cold shortening. Frozen meat (VFCf), corresponding to the part of the cut, was more tender than conventional chilled but tougher than meat of the inner part of muscle very fast chilled (VFCi).

CONCLUSION

Very fast chilling (VFC) causes important changes in the biochemical parameters related to meat tenderness: a higher ultimate pH, a slightly faster release of calcium and as a result of both and maybe other unknown effects, an enhacement of the proteolytic activity of calpains.
Very fast chilled meat was significantly more tender than meat conventionaly chilled.
The most important open question is now whether freezing of the outer part of the piece cut is related to meat tenderisation, or if freezing may be avoided by existing chilling technology.

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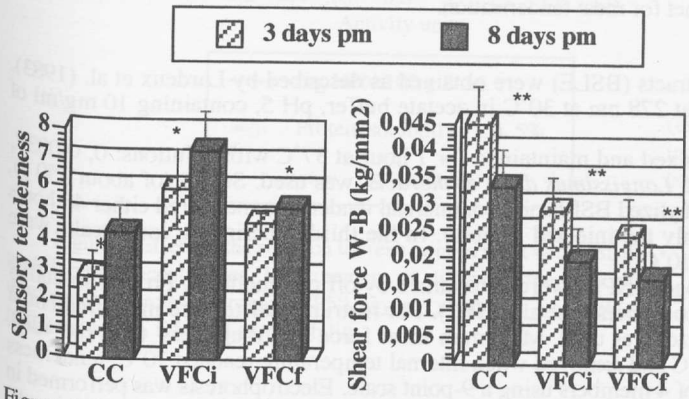

Figure 1: Instrumental and sensory analysis of meat texture subjected to conventional and very fast chilling *p<0.05 and ** p< 0.01. (Experiment 3).

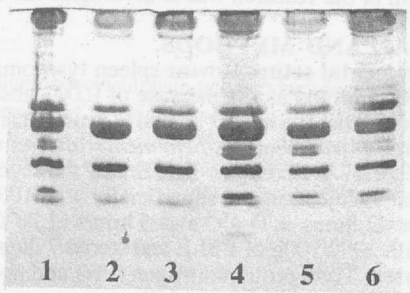

Figure 2: SDS-PAGE of myofibrillar proteins. **1**.- VFC rigor, **2**.- CC- rigor, **3**.- CC 3 d pm, **4**.- VFC 3 d pm, **5**.- VFC 8 d pm and **6**.- CC 8 d pm. (Experiment 3).

Table 1: Biochemical effects on *M. sternomandibularis* of chilling either in a -80°C freezer (VFC) or in cold room at 1±1°C (CC).

Experiment 1	Sarcomere length (μm) Rigor	pH Rigor	Ca++ concentration (μM) 5 hours pm	Ca++ concentration (μM) 14 days pm	Calpain activity 24 hours pm (UI/g) μ-calpain	Calpain activity 24 hours pm (UI/g) m-calpain	Shear force (kg/mm^2) 14 days pm
Conventional	1.64±0.09 a	5.6±0.04 a	0.81± 0.08 a	171± 4.33 a	0.022	0.033	0.092±0.021 a
Very fast chilling	1.58±0.12 a	5.7±0.03 b	1.42± 0.04 b	156± 4.33 b	-	0.029	0.109±0.023 a

UI/g is the rise of 0.001 units of optical density per gram. a,b Mean values in the same column are significantly different when accompanied by different letters (p<0.05).

Table 2.1: Biochemical effects on *M. sternomandibularis* of chilling either in a glycol bath at -6°C (VFC)or in cold room at 1 ±1°C (CC).

Experiment 2	Sarcomere length (μm) Rigor	pH Rigor	Calpain activity (UI/g) 2.5 hours pm μ-calpain	Calpain activity (UI/g) 2.5 hours pm m-calpain	Calpain activity (UI/g) 2.5 hours pm calpastatin	Calpain activity (UI/g) 3 days pm μ-calpain	Calpain activity (UI/g) 3 days pm m-calpain	Calpain activity (UI/g) 3 days pm calpastatin
Conventional	1.70±0.08 a	5.60±0.05 a	0.020	0.032	0.019	0.003	0.018	0.016
Very fast chilling	1.72±0.06 a	5.75±0.03 b	0.003	0.030	0.018	-	0.016	0.014

UI/g is the rise of 0.001 units of optical density per gram. a,b Mean values in the same column are significantly different when accompanied by different letters (p<0.01).

Table 2.2: Instrumental and sensory evaluation of *M. sternomandibularis* tenderness of chilling either in a glycol bath at -6°C (VFC)or in cold room at 1 ±1°C (CC).

Experiment 2	Shear force (kg/mm^2) 10 days pm	Sensory tenderness 10 days pm
Conventional	0.178±0.049 a	1.5±0.50 a
Very fast chilling	0.201±0.037 a	2.5±0.52 b

a,b Mean values in the same column and relating to each parameter are significantly different when accompained by different letters (p<0.05).

Table 3: Biochemical effects on *L. thoracis et lumborum* of chilling either in a liquid N_2 and CO_2 atmosphere at - 82.8°C or in cold room at 1±1°C

Experiment 3	Sarcomere length (μm) Rigor	pH Rigor	Ca++ concentration (μM) 4 hours pm	Ca++ concentration (μM) Rigor	Ca++ concentration (μM) 3 days pm
Conventional	1.35±0.06 a	5.45±0.03 a	1.09±0.068 a	1.47±0.087a	1.87±0.098a
Very fast (inner)	1.80±0.03 b	5.65±0.06 b	1.30±0.065 b	1.87±0.105b	2.04±0.127b
Very fast (frozen)		5.50±0.04 a	2.70±0.007 c	2.90±0.022c	3.56±0.042c

a,b Mean values in the same column and relating to each parameter are significantly different when accompained by different letters (p<0.05).