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Can calcium chloride injection facilitate the ageing-derived improvement in the quality of meat from culled dairy cows?

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ABSTRACT

This study investigated whether the positive effects of ageing on tenderness of meat from culled dairy cows can be facilitated by $CaCl_2$. Injections of 250 mM $CaCl_2$ solution (10% wt/wt) were performed on *Longissimus dorsi* samples from 32 7-yrs old cows. Samples were vacuum packaged and aged for 0, 1, 3, 5 and 7 days. Ageing alone produced lighter and less red meat with lower shear force, higher myofibrillar fragmentation and tenderness scores but also elevated lipid oxidation. For most traits investigated, $CaCl_2$-injected meat exhibited similar ageing effects, but drip loss increased with age. The $CaCl_2$-injected meat had a lower shear force and myofibrillar fragmentation increased more rapidly, but drip loss, off-flavour scores, colour stability and oxidative stability were inferior to untreated meat. Overall, it was found possible to accelerate tenderisation of such meat with $CaCl_2$, but only at the cost of adverse effects in some other quality traits.

1. Introduction

Tenderness is considered to be the most important beef quality trait with regard to eating quality (Miller, Carr, Ramsey, Crokett, & Hoover, 2001; Huff-Lonergan, Zhang, & Lonergan, 2010). In adult animals, age is a major limitation for valuable cuts to be tender enough to be marketed. A common means for tenderisation of meat is ageing for days or weeks under refrigerated conditions. This allows the breakdown, under hygienic conditions, of some of the complex proteins present in the muscle by myofibrillar enzymes like cathepsins and calpains (Goll, Tompson, Li, Wei, & Cong, 2003). The calpain system consists of two Ca-dependent enzymes, calpain I and calpain II, and a specific inhibitor, the calpastatin. Calpain II is mostly found in the cytosol, whereas calpain I is typically bound to the myofibril (Xu et al., 2009). The proteolytic systems present in the muscle include proteasomes, especially a multicatalytic proteinase complex called 20S proteasome (Dransfield, Etherington, & Taylor, 1992; Koohmaraie, 1996; Koohmaraie & Geesink, 2006; Kemp, Sensky, Bardsley, Buttery, & Parr, 2010). Proteasomes may be responsible for the final tenderness (Bernard et al., 2007; Kemp et al., 2010). Apart from ageing, other measures for tenderisation also rely on ways to facilitate enzymatic myofibrillar proteolysis. As the activity of the calpain system depends on Ca ion concentration (Steen, Claeys, Uytterhaegen, De Smet, & Demeyer, 1997; Goll et al., 2003), infusing a $CaCl_2$ solution into whole carcasses or cuts of meat improves tenderness (e.g., Wheeler, Koohmaraie, Lansdell, Siragusa, & Miller, 1993; Eilers et al., 1994; Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2003; Jaturasitha, Thirawong, Leangwunta, & Kreuzer, 2004). Other salts may be also effective in enhancing the tenderness of the meat from mature cows (Hoffman, 2006; Weber et al., 2013).

As longer-term ageing, which is also efficient in the tenderisation of culled cow beef (e.g. Stelzleni, Johnson, & Thrift, 2008; Franco, Bispo, González, Vázquez, & Moreno, 2009; Vitale, Pérez-Juan, Lloret, Arnau, & Realini, 2014), is associated with increasing costs and energy expenditure for chilling, a combination of tenderisation methods seems attractive. However, it remains unclear whether combining these methods, targeting the same process (i.e. facilitating the activity of the calpain system), is really more efficient than applying only one of the measures. Additionally, even though important, tenderness is not the only criterion determining purchasing decision, and undesired side-effects might render these attempts useless.

Ageing of the meat is associated with a reduction of colour stability and the risk of an increase in oxidative off-flavour. Likewise, there are some indications that extra chloride may induce discolouration (Kerth, Miller, & Ramsey, 1995) and may reduce the water-holding capacity of the meat (Jaturasitha et al., 2004). A large number of studies have investigated the effects of ageing and of $CaCl_2$ injection. However, apart from one small-scale study with four 6 to 7 yrs old steers (Kong, Diao, & Xiong, 2006), the concept of the combination of ageing with $CaCl_2$ injection of beef has so far, to the knowledge of the authors, only been described in a few experiments with growing-finishing animals (Wheeler, Koohmaraie, & Shackelford, 1997; De Moura, Luchiari,

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Nardon, & Razook, 1999; Cao et al., 2012), where rather tender beef could be expected anyway.

The hypotheses tested in the present study, therefore, were that (i) the effects of ageing and $CaCl_2$ injection on meat tenderness are at least additive or even associative, and (ii) the combination of ageing and $CaCl_2$ treatments can be performed with few undesired side-effects on flavour impression and acceptability, water-holding capacity as well as colour and oxidative stability of the beef. Underlying these hypotheses was the assumption that the combined approach tested might be particularly efficient for tenderisation of inherently tough beef from adult cattle like culled cows.

2. Materials and methods

2.1. Sample collection and treatment

Thirty-two 7-yrs old crossbred cows (75% Holstein-Friesian × 25% indigenous *Bos indicus*) originating from a local commercial farm were included in the present study. The cows had received maize silage ad libitum and 6 kg/hd per day of a commercial concentrate containing 160 g crude protein/kg. They free access to drinking water and a salt licking block. They were slaughtered at a commercial slaughter house according to current industry procedures over four slaughter days, being stunned using a captive bolt stunner and dressed according to commercial practices. Within 45 min of exsanguination, the entirety of the *longissimus dorsi* muscles (LD) was removed without bones from each carcass side, trimmed of all external fat and accessory muscles. Then, for the $CaCl_2$ injection, granular, food grade $CaCl_2$ was dissolved in distilled water to obtain a concentration of 250 mM. Following Jaturasitha et al. (2004), this concentration was chosen based on previous studies with beef where a significant tenderisation was found (members of a trained test panel stated that bitter off-flavours only occurred with 400 mM). The $CaCl_2$ solution was injected at up to 110% of wet meat weight with a hand stitch pump to the LD originating from the left side of the carcass. The entire LD (control as well as injected) was then cut into 25 steaks of 2.54 cm (1 in.) thickness (five for each of the five ageing times). All samples were then vacuum-packaged and kept in a holding cooler either for 0 or 1, 3, 5 and 7 days at 2 °C. At the end of the ageing periods, the vacuum-packaged steaks were frozen and stored at −20 °C until analysis, except for those designed for colour measurements which were left at 4 °C.

2.2. Shear force and myofibrillar fragmentation index

Meat samples were heated at 80 °C in plastic bags in a water bath (Korimat model 120/1.6, Christian Wanger, Esslingen, Germany) until the internal core temperature reached 70 °C, as monitored by a data logger and a copper thermocouple (Consort T851, Cohasset, MA, USA) inserted into the geometric centre of the steak. The samples were allowed to cool to 25 °C, and then maximal shear force and shear energy were measured using a Warner-Bratzler shear blade mounted on a texture analyser (model TA.XT plus, Stable Micro System Ltd., London, UK). Five cores from each steak with a diameter of 1.27 cm were drawn parallel to the longitudinal orientation of the muscle fibre and sheared perpendicular to this. A crosshead speed of 200 mm/min with a 5 kN load cell was used, calibrated to read over a range of 0 to 100 N (Jaturasitha et al., 2004).

The myofibrillar fragmentation index (MFI) was analysed using the procedure described by Olson, Parrish, and Stromer (1976), with slight modifications. Briefly, 4 g of LD was homogenised (Nissel AM-8 Homogenizer, Nihon Seiki Kaisha Ltd, Japan) for 30 s with 40 mL cold (2 °C) MFI-specific buffer (100 mM KCl, 20 mM KH_2PO_4, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM $NaNO_3$). With the aid of a funnel, the homogenate was poured into a 50 ml conical bottom centrifuge tube and centrifuged at 1,000 ×*g* for 15 min at 2 °C. The supernatant was discarded and the pellet was resuspended. The resuspension–centrifugation cycle was repeated for another two times and the final pellet was suspended in 10 mL of MFI buffer. In samples containing 0.5 mg/mL suspension, the absorbance at 540 nm was measured immediately with a spectrophotometer. The average of triplicate absorbance readings was multiplied by 150 to calculate the MFI.

2.3. Sensory evaluation

Following Bañón, Vila, Price, Ferrandini, and Garrido (2006), the steaks were first wrapped in aluminium foil in order to minimize moisture loss taking into account a certain change in meat structure due to the steam. The steaks were heated to an internal temperature of 70 °C in a convection oven pre-heated at 200 °C. The internal temperature of the samples was monitored by a data logger and thermocouple probe as described in Section 2.2. A panel consisting of nine trained members rated 20 samples per session over 16 sessions according to the standard outlined by AMSA (1995). The meat was evaluated for tenderness, juiciness, intensity of flavour (including both odour and taste), off-flavour intensity and overall acceptability using a nine-point scale (1 = extremely tough, dry, bland and unacceptable; 5 = average in all traits; 9 = extremely tender, juicy, intense and acceptable).

2.4. Water-holding capacity

Three different measures of water-holding capacity were applied. The first was ageing loss in the form of drip loss by suspending all steaks with a net placed into a plastic bag during ageing, and recording weight loss during the respective storage time intervals of 1, 3, 5, and 7 days. Thaw loss, being part of the ageing loss, was evaluated after different times of ageing by first freezing followed by thawing at 4 °C overnight. Finally, the meat samples cooked for later determination of shear force were cooled to room temperature and the surface dried with tissue paper in order to calculate cooking loss (initial weight minus final weight as the percentage of initial weight).

2.5. Meat colour

The aged, not previously frozen steaks were unpacked and allowed to bloom for 1 h at 4 °C. Then colour measurements were performed on three sites per steak using a Minolta Chroma Meter (CR 400, Minolta, Osaka, Japan; parameter settings: diffuse illumination, 0° viewing angle, measuring area, ø 11 mm) where lightness (L*), redness (a*), and yellowness (b*) were specified.

2.6. Oxidative stability of the muscular lipids

The susceptibility of the lipids to oxidation was assessed by the 2-thiobarbituric acid (thiobarbituric acid reactive substances, TBARS) assay (Rossell, 1994). Briefly, a 10 g portion taken from the individual LD was mixed with 30 ml distilled water for 2 min using a Moulinex household blender. A further 65 mL of distilled water was added, the pH adjusted to 1.44 with 2.5 ml of 4 M HCl, followed by drops of an antifoaming agent. Afterwards, the flask containing the sample was connected to the distillation apparatus. A 50 ml amount of the distillate was collected within 15 to 20 min, and 5 ml of this distillate allowed to react with 5 ml of TBA reagent. After cooling to room temperature, the absorbance was measured against a blank at 538 nm. The TBARS were calculated as 7.8 times the absorbance. Results were given as concentrations of mg malonaldehyde per kg of fresh meat.

2.7. Statistical analysis

Data were analysed by ANOVA with the SAS (2001; version 8) GLM procedure for the effects of days of ageing and $CaCl_2$ and the subsequent interaction. In cases where these effects were different at $P < 0.05$, mono-factorial approaches were applied for comparisons among