Food Chemistry 131 (2012) 73–78

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of calcium lactate on m-calpain activity and protein degradation under oxidising conditions

Yuan H. Brad Kim [a,b,*], Elisabeth Huff-Lonergan [a], Steven M. Lonergan [a]

[a] Muscle Biology Group, Department of Animal Science, Iowa State University, Ames, IA 50011, United States
[b] AgResearch Ltd., Ruakura Research Centre, Private Bag 3123, Hamilton 3240, New Zealand

ARTICLE INFO

Article history:
Received 31 March 2011
Received in revised form 7 July 2011
Accepted 9 August 2011
Available online 22 August 2011

Keywords:
Calcium lactate
Calpain
Protein degradation
Oxidation
High-oxygen modified atmosphere packaging

ABSTRACT

In two experiments, the effects of calcium lactate (CAL) on calpain activity were determined. In the model system, purified porcine skeletal muscle m-calpain was pre-incubated with various combinations of hydrogen peroxide (H_2O_2), calcium chloride, and/or different CAL concentrations. The m-calpain was activated by CAL, and the extent of m-calpain oxidation by H_2O_2 was significantly decreased with increasing CAL concentrations. In the muscle system, a beef *longissimus lumborum* (1 day postmortem) from each carcass ($n = 6$) was cut in half, randomly assigned to either 0.2 M CAL or water injection (WAT), and then packaged in a high-oxygen modified atmosphere. The CAL injected steaks resulted in less intact desmin and greater production of a 30-kDa troponin-T compared to the steaks in the WAT group. The CAL injection did not affect colour and lipid oxidation of steaks during display. These results suggest that CAL addition may improve tenderness of meat by enhancing activation of endogenous calpain and by protecting against calpain oxidation under oxidative conditions.

1. Introduction

Calpains play a significant role in postmortem proteolysis of cytoskeletal and myofibrillar proteins and thus affect the tenderisation of meat (Goll, Thompson, Li, Wei, & Cong, 2003; Koohmaraie, 1996). The most well identified members of the calpain proteinases in skeletal-muscle are μ- and m-calpain, which require both calcium and a reducing environment for activity resulting in postmortem proteolysis and tenderization (Goll et al., 2003). It is well documented that protein oxidation in meat can have a remarkable detrimental effect on meat protein functionality (Lund, Heinonen, Baron, & Estévez, 2011). *In vitro*, oxidative conditions diminish the activity of both μ- and m-calpain (Carlin, Huff-Lonergan, Rowe, & Lonergan, 2006). Moreover, oxidation of proteins in postmortem muscle can negatively affect proteolytic activity and autolysis of μ-calpain (Lametsch, Lonergan, & Huff-Lonergan, 2008; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004), and subsequently decrease the tenderness of meat (Rowe et al., 2004). In fact, the addition of calcium chloride to increase calpain activation in post rigor beef is more effective when there is a greater alpha tocopherol content in beef steaks (Harris, Huff-Lonergan, Lonergan, Jones, & Rankins, 2001). All of these observations suggest that proteolysis in meat can be improved by diminishing the effects of oxidation on the calpain proteinases.

Lactate has been widely used in the fresh meat industry as a non-meat ingredient. Injection enhancement of lactate in fresh meat increases the meat shelf-life by suppressing microbial growth (Brewer, Rostogi, Argoudelis, & Sprouls, 1995) and minimising oxidative quality defects, such as meat discolouration and off-flavour development mainly due to myoglobin- and lipid-oxidation (Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010a; Kim et al., 2009). Beef steaks enhanced with 0.1 M solution of calcium lactate had improved meat tenderness and palatability traits concurrent with acceptable display colour life and microbial inhibition (Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2003). What is not known, however, is the extent to which calcium lactate inclusion influences m-calpain activity and postmortem protein degradation under oxidative conditions. Although calcium lactate has free radical scavenging and antioxidant effects (Groussard et al., 2000; Kim et al., 2009), its antioxidant potential against calpain oxidation and subsequent impacts on proteolysis have not been published to our knowledge. Therefore, it can be hypothesised that calcium lactate addition to early postmortem muscle can both activate m-calpain and minimise oxidative inhibition of m-calpain under an oxidative condition such as a high-oxygen modified atmosphere (80% O_2 + 20% CO_2) packaging system (Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010b). Therefore, the objectives of this study were to (1) determine the effect of calcium lactate on m-calpain activity under various oxidising conditions *in vitro* and to (2) evaluate the effect of calcium lactate injection on postmortem proteolysis of beef steaks packaged in high-oxygen modified atmosphere.

* Corresponding author at: AgResearch Ltd., Ruakura Research Centre, Private Bag 3123, Hamilton 3240, New Zealand. Tel.: +64 7 838 5152; fax: +64 7 838 5625.
E-mail address: brad.kim@agresearch.co.nz (Yuan H. Brad Kim).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.08.033

2. Materials and methods

2.1. Experiment 1: Effects of calcium lactate on m-calpain activity under oxidising conditions in vitro

Porcine skeletal muscle m-calpain was purified following the procedures of Thompson and Goll (2000) with a few modifications (Carlin et al., 2006). One unit of m-calpain was defined as the amount of calpain required to increase the absorbance of the supernatant by one unit at 278 nm due to the release of trichloroacetic acid-soluble polypeptides by casein digestion (Koohmaraie, 1990). Purified m-calpain (66 U/mg) was pre-incubated in 40 mM Tris–HCl, at 23 °C for 10 min with various combination of 100 μM H_2O_2, 5 mM $CaCl_2$, and/or 5 mM calcium lactate (CAL) generating six treatments; (1) control, (2) H_2O_2, (3) $CaCl_2$, (4) CAL, (5) $CaCl_2$ + H_2O_2, and (6) CAL + H_2O_2. Then, the m-calpain activity of each pre-incubated treatment was measured in a standard casein assay under both reducing and non-reducing conditions (with and without 0.2% β-mercaptoethanol (MCE)) in triplicate (Koohmaraie, 1990). In order to determine the effects of lactate concentration on calpain activity, m-calpain (66 U/mg) was pre-incubated at the same conditions as above but with 50 μM H_2O_2 and with 5 mM $CaCl_2$, or CAL (5, 10, 15, and 20 mM).

2.2. Experiment 2: Effects of injection of calcium lactate on colour and lipid oxidation stability and postmortem proteolysis of beef longissimus

2.2.1. Raw materials and processing

Six market weight beef cattle [A-maturity; USDA (1997) Low Choice grade] were slaughtered at the Iowa State University Meat Laboratory. At 24 h postmortem, the *musculus longissimus lumborum* was removed from each side of a carcass, and were cut into 2.54 cm thick steaks by cutting perpendicular to the muscle length. The steak from the anterior portion of each muscle ($n = 6$) was cut in half. Immediately after cutting, one of the half was randomly assigned to a syringe injection (10%) with a solution containing 0.2 M calcium lactate (CAL, pH 6.8), and the other half was injected with distilled water (WAT). The injected steaks were placed in preformed trays (polypropylene/ethylene vinyl alcohol, 22.5 × 17.3 × 4.1 cm; Rock-Tenn.; Franklin Park, IL). Trays were put in a shrinkable bag (B620, 20.3 by 30.48 cm/an oxygen-transmission rate of 3 $cc O_2$/1 cm^3/m^2/24 h at 4.4 °C and 0% relative humidity and a water–vapour transmission rate of 0.5–0.6 g/254 cm^2/24 h at 37.8 °C and 100% relative humidity; Cryovac Sealed Air Corporation, Bolingbrook, IL), and packaged to a high-oxygen modified atmosphere (HiOx-MAP; 80% O_2/20% CO_2, Certified Standard within ±2%, Praxair, Inc. Specialty Gases; Cahokia, IL). HiOx-MAP was accomplished by using a packaging machine (Multivac C500, Koch Supplies Inc., Kansas City, MO) by applying vacuum, then flushing the package with the gas mixture, and sealing. Packages were displayed for 8 days at 1 °C under continuous fluorescent natural white light (Sylvania F40N, 3600 K, CRI = 86; Osram Sylvania, Danvers, MA) of 2150 ± 50 lux intensity. The gas composition of each package from the initial display (designated as day 1; 48 h postmortem) and end of display (designated as day 8; 9-day postmortem) was determined by using a headspace oxygen/carbon dioxide analyser (PBI Dansensor, Glen Rock, NJ) prior to an instrumental colour measurement.

2.2.2. Colour

The surface colour (CIE $L^*a^*b^*$) of steaks from the initial (day 1; 48 h postmortem) and at the end of display (day 8; 9-day postmortem) was measured using a HunterLab LabScan®XE Spectrophotometer (Illuminant A, 2.54 cm diameter aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA). Calibration was performed by using standard black and white tiles prior to the colour measurement. CIE $L^*a^*b^*$ values were used to calculate saturation index $[(a^{*2} + b^{*2})^{1/2}]$ and hue angle $[(b^*/a^*)^{\tan-1}]$ to evaluate colour intensity (saturation index) and discoloration (hue angle) (AMSA, 1991). Three different locations for each steak were scanned and averaged for statistical analyses.

2.2.3. pH determination

The pH for each steak sample during display was measured by a calibrated pH probe (Hanna 9025 pH/ORP metre; Hanna Instruments, Woonsocket, RI) on three different locations of each steak. The calibration of pH electrode was performed with standardised buffers (pH 4.0 and 7.0) prior to each measurement for steak samples.

2.2.4. Lipid oxidation

Lipid oxidation of steaks from taken at 48 h postmortem and at the end of display (day 8 of storage; 9-day postmortem) was determined by using the 2-thiobarbituric acid distillation method described by Tarladgis, Watts, Younathan, and Dugan (1960). The absorbance at 532 nm of meat samples that had been collected form a distillation process and reacted with 2-thiobarbituric acid were measured using a spectrophotometer. The absorbance value was used to calculate mg malonaldehyde per kg of sample by multiplying by a factor of 7.8 to obtain the thiobarbituric acid-reactive substances (TBARS) value. Duplicate TBARS measurements of sample were determined, and averaged for statistical analyses.

2.2.5. Whole muscle sample preparation for gel electrophoresis

Whole muscle sample preparation for Western blotting was conducted from each steak at the initial (day 1; 48 h postmortem) and end of display (day 8; 9-day postmortem) following the procedure of Lonergan, Huff-Lonergan, Rowe, Kuhlers, and Jungst (2001). Protein concentration was determined as described by Lowry, Rosebrough, Farr, and Randall (1951) using premixed reagents (Bio-Rad Laboratories, Hercules, CA). Gel samples were adjusted to 4 mg/ml of protein and were frozen and stored at −80 °C until used for analysis.

2.2.6. Western blotting

Western blotting for μ-calpain autolysis, troponin-T and desmin degradation was conducted as described by Kim et al. (2010b). Protein (20 μg) of protein per lane and the same reference was loaded onto each gel. After electrophoresis, the gels were transferred to polyvinylidene (PVDF) membranes (Millipore Corporation, Bedford, MA) according to the procedures of Kim et al. (2010b). After transfer, the blots were incubated at 4 °C with their respective primary antibody (desmin = 1:20,000 dilution with PBS-Tween, polyclonal rabbit anti-desmin antibody, V2022; Biomeda, Foster City, CA; troponin-T = 1:40,000 dilution with PBS-Tween, monoclonal anti-troponin-T antibody, JLT-12; Sigma Chemical Co., St. Louis, MO; μ-calpain = 1:10,000 dilution with PBS-Tween, monoclonal anti-μ-calpain antibody, MA3-940; Affinity Bioreagents, Inc., Golden, CO), and their respective secondary antibody (μ-calpain and troponin-T = 1:10,000, and 1:30,000 dilution with PBS-Tween, goat anti-mouse-HRP, No. A2554; Sigma Chemical Co., St. Louis, MO; desmin = 1:20,000 dilution with PBS-Tween, goat anti-rabbit-HRP, No. A9169; Sigma Chemical Co., St. Louis, MO). Protein bands were detected using a chemiluminescent detection kit (ECL, Amersham Pharmacia Biotech), and were quantified by densitrometry using ChemiImager 5500 (Alpha Innotech, San Leandro, CA) and Alpha Ease FC (v. 2.03; Alpha Innotech). The bands for the degradation product of troponin-T (30-kDa band) and intact desmin were expressed as a relative ratio compared to the reference sample (bovine *m. longissimus* packaged in HiOx-MAP for 9 days; 10-day postmortem).