Meat Science 91 (2012) 247–254

Contents lists available at SciVerse ScienceDirect

Meat Science

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

Profile of biochemical traits influencing tenderness of muscles from the beef round

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ARTICLE INFO

Article history:
Received 4 January 2011
Received in revised form 24 January 2012
Accepted 25 January 2012

Keywords:
Round
Tenderness
Beef

ABSTRACT

The objective of this study was to define the biochemical differences that govern tenderness and palatability of economically important muscles from the beef round using cuts with known tenderness differences. At 24 h postmortem, the longissimus dorsi (LD), gracillus (GR), adductor (AD), semimembranosus (SM), sartorius (SAR), vastus lateralis (VL), and vastus intermedius (VI) muscles were removed from ten market weight beef cattle. Sensory and biochemical characteristics were determined in each cut and compared with the LD. The GR, SAR and VI had sensory traits similar to the LD while the SM, AD and VL differed. The GR, SAR, AD, and SM all had multiple biochemical characteristics similar to the LD, while the VI and AD had numerous biochemical differences. While no one biochemical characteristic can be used to predict tenderness across all muscles, analysis of the biochemical characteristics revealed that in most beef round cuts postmortem proteolysis provided a good indication of the tenderization occurring during aging.

1. Introduction

Consumers rate tenderness as the primary sensory trait considered when they are making purchasing decisions (Mennecke, Townsend, Hayes, & Lonergan, 2007), and are willing to pay a premium in order to purchase a consistently tender product (Boleman et al., 1997; Miller, Carr, Ramsey, Crockett, & Hoover, 2001; Platter et al., 2005). Definition of molecular features that affect beef tenderness is necessary to develop robust methods to predict and define beef quality.

The muscles of the round are particularly prone to being less tender than the higher value cuts of the wholesale loin and rib. This generalization has historically limited the merchandizing of the round. Recently, however, approaches to merchandise cuts from individual muscles of the beef chuck have shown that some cuts can be merchandized as higher value cuts. While the muscles of the round originate from a similar location, they have decidedly different fiber types and sensory properties (Kirchofer, Calkins, & Gwartney, 2002; McKeith, Devol, Miles, Bechtel, & Carr, 1985). In addition to this, the muscles of the round traditionally have received similar aging procedures. However, these muscles have distinctly different biochemical characteristics that will affect the quality traits of the cut.

It is accepted that the rate and extent of postmortem proteolysis of key proteins in meat can dictate the development of tenderness, especially in the often characterized cuts from the longissimus dorsi. The calpain system, specifically μ-calpain, has been found to be responsible for the majority of postmortem proteolysis of muscle proteins associated with tenderness (Geesink, Kuchay, Chishti, & Koohmaraie, 2006; Huff-Lonergan, Zhang, & Lonergan, 2010). Investigating muscle differences for μ-calpain and calpastatin activity will allow identification of differences in rate of tenderization and final tenderness of these individual muscles. One factor affecting calpain activity is the rate of early postmortem pH decline. This influences the rate of μ-calpain activity and autolysis and may play a pivotal role in regulating early postmortem proteolysis and ultimately postmortem tenderization (Melody et al., 2004; Pomponio, Ertbjerg, Karlsson, Costa, & Lametsch, 2010). In addition to postmortem proteolysis and pH, connective tissue, muscle fiber type, and sarcomere length can play a large role in postmortem tenderization. Muscle fiber type can play a key role in the rate and extent of the postmortem pH decline (Choi, Ryu, & Kim, 2006). The amount of connective tissue and sarcomere shortening during rigor development are primarily responsible for establishing the background toughness prior to aging (Koohmaraie, Doumit, & Wheeler, 1996; Koohmaraie & Geesink, 2006; Wheeler & Koohmaraie, 1994). Practical considerations, including a slow chilling rate in the center of large muscles like the semimembranosus can also negatively affect protein solubility and thus tenderness and color of fresh beef (Kim, Lonergan, & Huff-Lonergan, 2010).

Understanding the extent to which biochemical changes contribute to the tenderness can improve utilization of these cuts. The objective of this study was to use economically important cuts from the beef round with known differences in tenderness to define the underlying biochemical differences that govern tenderness and palatability of these cuts. Specific changes targeted include the factors that

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doi:10.1016/j.meatsci.2012.01.022

regulate the calpain system as well as the products of protein degradation in postmortem beef.

2. Materials and methods

2.1. Sample collection

Ten market weight English breed cross-bred beef cattle that were under 20 months of age, were slaughtered at the Iowa State University Meat Laboratory using normal, approved humane slaughter procedures. Slaughter took place on five different dates with animals slaughtered in pairs. Prior to slaughter, animals were stunned using a captive bolt stunner. No electrical stimulation was used. Carcasses were chilled at 4 °C for 24 h prior to grading. All cattle had quality grades of either choice or select. Muscles were removed from both sides of the carcasses at 24 h postmortem. Muscles removed included the longissimus dorsi (LD; reference muscle) and the following muscles from the round: gracillus (GR), adductor (AD), semimembranosus (SM), sartorius (SAR), vastus lateralis (VL), and vastus intermedius (VI). Muscles were cut into two 2.54 cm thick and one 0.64 cm thick steaks per aging period for a total of 12 steaks per muscle. Steaks were cut perpendicular to the long axis of the muscle and were individually vacuum packaged. All steaks (2.54 cm and 0.64 cm thick) were aged at 4 °C for a total of one, three, seven or 14 days postmortem. The 2.54 cm thick steaks were frozen in their vacuum packages at the end of their respective aging periods and were used for star probe and sensory analysis. The 0.64 cm thick steaks were used for biochemical analysis.

2.2. Measurement of pH

A Hanna 9025 pH/ORP meter (Hanna Instruments, Woonsocket, RI) was used to measure pH of each muscle at 24 h postmortem. The pH probe was calibrated using two buffers (pH 4.0 and 7.0), and maintenance of calibration was monitored between samples.

2.3. Measurement of calpastatin activity

Calpastatin activity was determined for each muscle at 24 h postmortem (Koohmaraie, Shackelford, Wheeler, Lonergan, & Doumit, 1995; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004). One unit of calpastatin activity was defined as the ability to inhibit one unit of m-calpain activity (Koohmaraie, 1990). Activity was recorded as units/gram of muscle tissue.

2.4. Whole-muscle sample preparation for gel electrophoresis

Samples were taken from each muscle (0.2 g) at each aging period for western blotting to determine autolysis of μ-calpain and postmortem degradation of desmin and troponin-T. Whole-muscle protein samples were extracted and samples were prepared for gel electrophoresis (Lonergan, Huff-Lonergan, Rowe, Kuhlers, & Jungst, 2001). Protein concentration was determined using the method described by Lowry, Rosebrough, Farr, and Randall (1951) using premixed reagents (Bio-Rad Laboratories, Hercules, CA). Protein samples were adjusted to a concentration of 6.4 mg/mL of protein using the whole muscle extraction buffer. Wang's tracking dye (3 mM EDTA, 3% SDS [wt/vol], 30% glycerol [vol/vol], 0.001% pyronin Y [wt/vol], 30 mM Tris–HCl, pH 8.0) and 2-mercaptoethanol (MCE) were added to the adjusted protein sample at the ratio [vol/vol] of 0.5 to 1 to 1 (Wang's tracking dye) and 0.1 to 1 (MCE) to bring each sample to a final concentration of 4 mg/mL. The resulting gel samples were frozen and stored at −80 °C until subsequent analysis.

2.5. SDS-PAGE electrophoresis and western blotting

Gel samples (whole muscle extract, tracking dye, and MCE) from each muscle and aging period were used to determine μ-calpain autolysis and troponin-T degradation. Only samples from one, seven, and 14 days postmortem were used to determine desmin degradation. Proteins were resolved on acrylamide separating gels (acrylamide: *N*,*N*′-bis-methylene acrylamide = 100:1 [wt/wt], 0.1% SDS [wt/vol], 0.05% *N*,*N*,*N*′*N*-tetramethylethylenediamine (TEMED), 0.05% ammonium persulfate [wt/vol], and 0.5 *M* Tris–HCl, pH 8.8) for SDS-PAGE and western blotting. A 5% acrylamide stacking gel (acrylamide: *N*,*N*′-bis-methylene acrylamide = 100:1 [wt/wt], 0.1% SDS [wt/vol], 0.125% TEMED, 0.075% ammonium persulfate [wt/vol], and 0.125 *M* Tris–HCl, pH 6.8) was used on all of the acrylamide gels. Gels (10 cm × 10.5 cm) for μ-calpain autolysis, desmin and troponin-T were run on SE 260 Hoefer units (Hoefer Scientific Instruments). Western blotting for μ-calpain autolysis, desmin and troponin-T was performed on 9%, 10%, and 12% acrylamide gels, respectively. Western blots for μ-calpain autolysis, desmin and troponin-T were completed using the method described by Rowe et al. (2004).

Primary antibodies for μ-calpain autolysis, desmin and troponin-T were monoclonal anti-μ-calpain antibody (MA3-940; Affinity Bioreagents, Inc., Golden, CO), polyclonal rabbit anti-desmin (V2022; Biomedia, Foster City, CA), and monoclonal anti-troponin-T antibody (JLT-12; Sigma Chemical Co., St. Louis, MO), respectively. All membranes were incubated in primary antibody at 4 °C overnight.

The secondary antibody for μ-calpain autolysis and troponin-T blots was goat anti-mouse-HRP (catalog No. A2554; Sigma Chemical

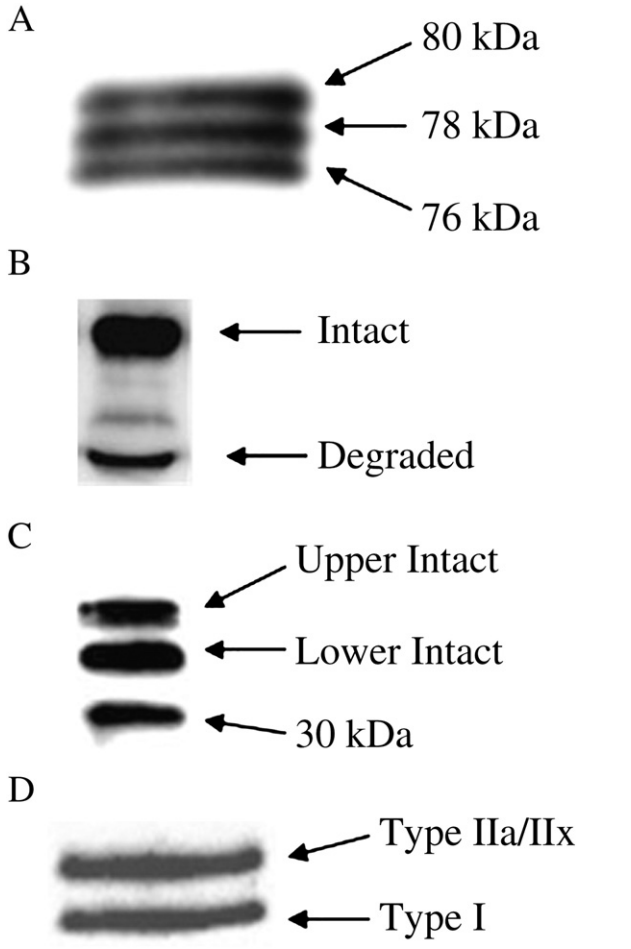

Fig. 1. Examples of banding pattern in a) μ-calpain, b) desmin, and c) troponin-T western blots and d) SDS PAGE analysis of myosin heavy chain isoforms.