Meat Science 82 (2009) 324–330

Contents lists available at ScienceDirect

Meat Science

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

Pre-rigor infusion with kiwifruit juice improves lamb tenderness

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ARTICLE INFO

Article history:
Received 16 September 2008
Received in revised form 4 February 2009
Accepted 4 February 2009

Keywords:
Lamb
Infusion
Kiwifruit
Tenderness
Actinidin
Calpain

ABSTRACT

The ability of pre-rigor infusion of kiwifruit juice to improve the tenderness of lamb was investigated. Lamb carcasses were infused (10% body weight) with fresh kiwifruit juice (Ac), water (W) and a non-infusion control (C) treatment. Infusion treatment had no effect on lamb hot carcass weight, cold carcass weight and chilling evaporative losses. The infused treatment carcasses of Ac and W had lower ($P < 0.05$) pH values than C carcasses during the initial 12 h post-mortem. The LD muscles from Ac carcasses were more tender with significantly lower shear force ($P < 0.001$) compared with C and W carcasses during the six days following infusion with the kiwifruit juice. The enhanced proteolytic activity ($P = 0.002$) resulting from the infused kiwifruit juice in Ac carcasses was associated with significant degradation of the myofibrillar proteins, appearance of new peptides and activation of m-calpain during post-mortem ageing. Thus, kiwifruit juice is powerful and easily prepared meat tenderizer, which could contribute efficiently and effectively to the meat tenderization process.

1. Introduction

Consumer acceptance of meat is strongly influenced by the eating quality including tenderness, juiciness and flavour (Lawrie, 1998). Surveys have shown that tenderness is the most important palatability trait for consumers (Shackelford et al., 2001). The red meat industry needs to produce high quality meat of consistent tenderness to increase consumer confidence and encourage further purchase of meat products (Boleman et al., 1997).

Several tenderizing solutions (Farouk, Price, & Salih, 1992; Hunt et al., 2003), plant proteases (Ashie, Sorensen, & Nielsen, 2002; Lewis & Luh, 1988; Wada, Suzuki, Yaguti, & Hasegawa, 2002) and ions (Koohmaraie & Shackelford, 1991; Koohmaraie, Shackelford, & Wheeler, 1998; Murphy & Zerby, 2004) have been used to accelerate meat tenderization through injection, infusion or marinating. Papain (Ashie et al., 2002; Schenkova et al., 2007) and calcium chloride (Ilian et al., 2004; Koohmaraie, Crouse, & Mersmann, 1989; Koohmaraie et al., 1998) have been the most studied and are probably the most effective tenderizing agents. However, papain has a tendency to over-tenderize the meat surface, leading to undesirable "mushy" meat (Ashie et al., 2002; Lewis & Luh, 1988), which has limited its use as a commercial meat tenderizer. Although the infusion of $CaCl_2$ solution can improve meat tenderness (Koohmaraie et al., 1989, 1998), calcium ions reduce the colour stability of fresh meat and decrease the product shelf life (Bekhit et al., 2005; Hunt et al., 2003). Actinidin, a cysteine protease from kiwifruit, was reported to have a mild tenderizing effect on beef (Lewis & Luh, 1988; Wada, Hosaka, Nakazawa, Kobayashi, & Hasegawa, 2004; Wada et al., 2002). Earlier research used purified actinidin and marinating techniques (Lewis & Luh, 1988; Wada et al., 2002). The current study evaluated the tenderizing effect of pre-rigor infusion of lamb carcasses with fresh kiwifruit juice with the ultimate aim of using fresh kiwifruit juice as a commercial meat tenderizer.

2. Materials and methods

2.1. Animals, infusion treatments, kiwifruit juice and sample preparation

A total of 18 lambs (12 months old, average live weight 41.0 ± 0.85 kg) were randomly selected from the Lincoln University farm, and were divided into three groups (6 lambs in each group/treatment). Three treatments were used: 50% kiwifruit fresh juice supernatant infusion (Ac), water infusion (W), and no infusion as a control (C). The kiwifruit juice was made fresh each day. After the kiwifruit was pressed with a bladder press, the juice was filtered through a fine filter cloth and centrifuged at 8500*g* for 20 min at 4 °C. The supernatant was diluted with an equal volume of distilled water for infusion.

Three animals from each treatment were slaughtered and processed on each of two consecutive days. Animals were slaughtered with the standard captive bolt stunning procedure followed by exsanguination, infusion (for Ac and W carcasses) and dressing.

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doi:10.1016/j.meatsci.2009.02.003

The infusion procedures were as described by Ilian et al. (2004) and the volume of infusion solution was 10% of body weight. After dressing, the carcasses from the various experimental treatments were weighed (hot carcass weight) and kept at room temperature (10 °C) for 5 h before they were moved to a chiller at 2 °C and stored for 6 days. The chilling evaporative loss was calculated as the difference between hot carcass weight and cold carcass weight determined 1 day after they had been placed in the chiller.

Meat quality measurements, including shear force and degradation of myofibrillar proteins, were made on the longissimus dorsi (LD) muscle and hind leg cut during the 3 weeks post-mortem. During the 6 days post-mortem, samples (5 cm thick portions) were taken from the carcass right loin at 0.5 h, 5 h, 12 h, 1 day, 2 days, 4 days, 6 days post-mortem. The carcass left loin was excised at 24 h post-mortem and a sample was taken, vacuum packed and stored at 2 °C for 3 weeks post-mortem analysis. Leg chop samples (2.5 cm thick portions) taken from the right hind leg were prepared at 1 day post-mortem, individually vacuum packed and stored at 2 °C. At 1 day, 2 days, 4 days, 6 days and 3 weeks post-mortem, the leg chop samples were taken from the chiller and used for the experiments. The leg chop samples were not homogeneous as they were made up of several muscles including rectus femoris, sirloin tip, semimembranosus, adductor, biceps femoris and semitendinosus (Forrest, Alberle, Hedrick, Judge, & Merkel, 1975). At sampling, the samples were frozen rapidly in liquid nitrogen to stop biochemical changes, and then individually vacuum packed and stored (approximately 1 month) at −30 °C until analyses.

2.2. pH and temperature

The pH and temperature profiles of lamb carcasses during onset of rigor were determined as described by Bekhit et al. (2005). The pH and temperature were measured every 30 min during the initial 8 h post-mortem, and then at 12 h, 1 day and 2 days post-mortem in the LD using an InLab 427 pH combination puncture electrode (Mettler–Toledo Process Analytical Inc., Wilmington, MA, USA) attached to a Hanna HI 9025 pH meter (Hanna Instruments Inc., Woonsocket, USA).

2.3. Shear force measurements

Frozen meat samples from the LD excised at 5 h, 12 h, 1 day, 2 days, 4 days, 6 days and 3 weeks post-mortem, and leg chop samples obtained at 1 day, 2 days, 4 days, 6 days and 3 weeks post-mortem were thawed overnight at 2 °C and cooked individually in plastic bags immersed in a water bath at 90 °C until they reached an internal temperature of 75 °C. A Fluke type K thermocouple attached to Fluke 52 m (John Fluke MFG CO., Washington, USA) was pushed into the centre of the meat samples during cooking. Each cooked steak was cooled on ice, and then cut into 8–10 strips (10 × 10 mm square) parallel to the muscle fibre direction. The meat strips were placed separately in a MIRINZ tenderometer (AgResearch MIRINZ, Hamilton, New Zealand) and the shear force was measured in Newtons (N).

2.4. Actinidin activity assay

Protease activity in meat sample extracts and kiwifruit juice was assayed using CBZ-Lys-ONP as a substrate (Lewis & Luh, 1988). Minced meat samples (1 g) from the LD excised at 0.5 h were homogenized with 5 mL of enzyme assay buffer using a Polytron homogeniser (Type PT-MR 3100, Kinematica AG, Littau, Switzerland) at 15,000 rpm for 20 s and then centrifuged at 37,000g for 30 min at 4 °C. The supernatant was weighed and stored in the ice box ready for the assay.

A 2.98 mL aliquot of assay buffer (0.2 mM EDTA, 1.6% acetonitrile in 0.07 M potassium phosphate buffer, pH 6.95) was added to 50 μL of the substrate solution (10 mM CBZ-Lys-ONP in 19:1 acetonitrile to water). After the initial degradation rate of the substrate had been measured for 30 s at 348 nm, 50 μL of kiwifruit juice solution or meat sample supernatant was added and the rate of substrate degradation was measured for a further 30 s. The amount of *p*-nitrophenol produced was determined by reference to a standard curve. The initial non-enzymatic hydrolysis rate of substrate was subtracted from the enzyme activity rate and one unit of proteolytic activity was defined as the amount of enzyme which produced 1 μmol of product (*p*-nitrophenol) per min.

2.5. SDS–PAGE of sarcoplasmic and myofibrillar proteins

Sarcoplasmic and myofibrillar fractions of LD proteins from samples at 0.5 h, 1 day and 6 days post-mortem time were separated according to the procedure described by Ilian et al. (2004). Muscle tissue (2 g) was cut into small pieces and homogenized in three volumes of a homogenizing buffer (100 mM Tris–HCl, 5 mM EDTA, 0.05% β-mercaptoethanol, pH 8.3) at 2 °C for 30 s at 13,500 rpm using a Polytron homogeniser (Type PT-MR 3100, Kinematica AG, Littau, Switzerland). The homogenate was centrifuged at 5500*g* for 30 min at 4 °C. After centrifuging, 1 mL of the supernatant containing the sarcoplasmic protein fraction was decanted into an eppendorf tube and was frozen for further use in zymographic PAGE analysis. The rest of the supernatant was discarded. The pellet was washed twice with 15 mL of homogenizing buffer and was centrifuged at 2300*g* for 10 min at 4 °C. The washed pellet was then re-suspended in 15 mL SDS–PAGE sample buffer (50 mM Tris–HCl pH 6.8, 1% SDS, 2% MCE, 3% glycerol) and was heated at 80 °C in a water bath for 3 min. The hot sample was vortexed, filtered through Whatman No. 1 filter paper and then 1 mL of the separated solution was stored at 4 °C for myofibrillar protein analysis using SDS–PAGE.

The protein concentration of the various fractions was determined according to the method of Karlsson, Ostwald, Kabjorn, and Anderson (1994). The SDS–PAGE procedure was as described in the Mini-PROTEAN 3 Cell Instruction Manual, BioRad. Samples (20 μg) were separated on a 12% (w/v) polyacrylamide gel at 150 V for 70 min in a Tris–glycine buffer. The gels were stained for 1 h with Gelcode Blue (Pierce, USA) and destained in distilled water overnight to reveal the bands.

2.6. Zymographic PAGE analysis

The zymography was executed according to the procedure described by Ilian et al. (2004). A 10% polyacrylamide gel co-polymerised with 0.05% casein was pre-run with a pH 8.3 buffer containing 25 mM Tris, 192 mM glycine, 1 mM EGTA and 1 mM DTT for 15 min at 125 V at 4 °C. Then 10 μL each of kiwifruit juice (50% concentration), μ-calpain and m-calpain, and 12 μL sarcoplasmic protein samples (refer to section 2.5 for details of sample preparation) were loaded and run for 2.5 h at 125 V at 4 °C. Subsequently the gels were incubated overnight at room temperature with slow shaking in buffer (20 mM Tris–HCl, 10 mM DTT, pH 7.4) with or without 20 mM calcium chloride. Gels were rinsed thoroughly with distilled water 3 times for 10 min each, and then stained with Gelcode Blue (Pierce, USA) for at least 1 h.

2.7. Analysis of data

Data of live weight, hot carcass weight, cold carcass weight and chilling evaporative loss were tabulated in Microsoft Excel spreadsheets, means and standard error of means (SEMs) of the measurements were calculated by using Minitab software (Minitab version