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Freezing, thawing and aging effects on beef tenderness from *Bos indicus* and *Bos taurus* cattle

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ABSTRACT

The objective of this study was to determine the effects of freezing prior to aging on the meat tenderness of young Nellore and Aberdeen Angus bulls. Samples of the *longissimus thoracis* muscle were submitted to two treatments: conventional aging and freezing (−20 °C for 40 days) followed by thawing and aging periods. The meats were evaluated after 0, 7, 14 and 21 aging days (1 °C). Freezing increased ($P < 0.05$) purge, cooking loss and total exudate loss throughout aging. Nellore meats had greater total exudate loss and shorter sarcomere lengths ($P < 0.05$). Freezing increased proteolysis during aging in the meats of both breeds, but reduced shear force was found ($P < 0.05$) only in Aberdeen Angus meats and only at time zero. These results suggest that the meat tenderizing process by freezing prior to aging may contribute to meat tenderness in the first weeks of aging, but it is dependent on the animal breed.

1. Introduction

Cattle production is one of the most important sectors of Brazilian agribusiness and contributes substantially to production worldwide. Brazil has the largest commercial herd in the world and is the greatest exporter of beef and the second greatest meat producer (USDA, 2014). It is estimated that 80% of the Brazilian herd consists of Zebu (*Bos indicus*) breeds. These animals have a proven rusticity and adaptation to the tropical environment of Brazil, with Nellore maintaining 90% of this share (ABIEC, 2014). Nevertheless, the productive chain of Brazilian livestock is experiencing an increase in the introduction of taurine breeds (*Bos taurus*), such as Aberdeen Angus, mainly in the southern region. Here, low temperatures and pastures with increased nutritive value make the adaptation of these animals possible.

Among the genetic groups of cattle, *B. taurus* animals generally present more tender meat than *B. indicus* animals (Bressan, Rodrigues, Rossato, Ramos, & Gama, 2011; Shackelford, Koohmaraie, Miller, Crouse, & Reagan, 1991; Wheeler, Savell, Cross, Lunt, & Smith, 1990; Whipple et al., 1990), which can lead to decreased acceptance of Zebu meat by consumers. The lower tenderness of *B. indicus* meats is mainly due to the higher content of calpastatin present in their muscle, which inhibits the action of calpains, major proteases involved in the aging process (Koohmaraie, 1994; Koohmaraie & Geesink, 2006; Shackelford et al., 1991; Whipple et al., 1990).

There are indications that the application of a freezing process prior to aging can provide an alternative to reduce calpastatin activity, minimizing its effects and enhancing meat tenderness. Koohmaraie (1990) and Whipple and Koohmaraie (1992) reported greater susceptibility to the inactivation of calpastatin in beef when submitted to freezing temperatures, while the calpain activity remained relatively stable. These authors suggested that freezing and thawing of meat would increase the proteolytic activity of calpains during aging and, thus, meat tenderness.

Meat tenderness, measured by shear force, increases with freezing and thawing (Lagerstedt, Enfält, Johansson, & Lundström, 2008; Shanks, Wulf, & Maddock, 2002; Vieira, Diaz, Martínez, & García-Cachán, 2009), although some authors (Eastridge & Bowker, 2011; Hergenreder et al., 2013; Wheeler, Miller, Savell, & Cross, 1990) have observed no differences in tenderness between fresh and thawed meats. In most of these studies, however, the effects of freezing were evaluated after meat aging. When aging is conducted after freezing and thawing of meat, some studies (Carolino et al., 2009; Cohen, 1984; Crouse & Koohmaraie, 1990; Grayson, King, Shackelford, Koohmaraie, & Wheeler, 2014) have reported a positive effect on beef tenderness.

Although freezing prior to aging may be used to address Zebu beef tenderness problems, there has been no scientific report of these effects in *B. indicus* meat. Therefore, the objective of this study was to evaluate

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the effects of meat aging after frozen storage on the tenderness of the *longissimus thoracis* muscle of Aberdeen Angus and Nellore animals.

2. Material and methods

Experimental procedures were approved by the Ethics and Animal Welfare Committee of the Universidade Federal de Lavras (UFLA). The animals were raised in the outbuildings of the Department of Animal Science, and the experiment was conducted in the Laboratory of Meat Technology (LabCarnes) of the Department of Food Science.

2.1. Animals and sample preparation

Seventeen Nellore bulls (*B. indicus*) were purchased from three different beef cattle farms in Minas Gerais state, and they were sons of three purebred Nellore sires. Seventeen Aberdeen Angus bulls (*B. taurus*) were obtained from other three beef cattle farms in Rio Grande do Sul state (Southern Brazil), and they were sons of three Angus sires. All sires were chosen based on their prolificacy in the Brazilian beef herd. We used this procedure to work with animals that represent both breeds and avoid a possible sire effect. At 20 months of age the animals were transported to the Beef Cattle facility of the Federal University of Lavras, in Minas Gerais state. The diets were fed *ad libitum* and finished in feedlot at the same time (diet were based on corn silage and concentrate: ground corn grain, soybean meal and mineral mixture) for 112 days and slaughtered with average weight of 492 ± 61 kg. The animals were killed by cerebral concussion and bled by venisection of the jugular and carotid, following welfare and slaughter practices enacted by Brazilian legislation. Electrical stimulation has not been applied. After washing, each half-carcass was hung by the foramen pelvis and kept in a refrigerated room (4 °C) for 24 h. Then, the right *longissimus thoracis* muscle was removed from the 6th thoracic vertebra, and twelve 2.54 cm thick steaks were obtained (from the caudal end) and sequentially identified for treatment in the following manner (Fig. 1): the first steak and half of the second (experimental unit) were subjected to the conventional aging treatment (control); the other half of the second and the entire third steak were treated by freezing prior to aging (frozen–thawed). This process was repeated until up to four experimental units for each treatment were obtained. Each experimental unit (1.5 steaks) was identified, weighed and packaged under vacuum (Packer model BS420, R. Baião, Brazil) in nylon-polyethylene packages. The samples for conventional aging (control) were stored under refrigeration (1 ± 0.5 °C) in an environmental chamber (Model EL202, EletroLab, Brazil), for 0 (24 h *post mortem*), 7, 14 and 21 days. The samples subjected to freezing prior to aging (frozen–thawed) were frozen immediately after they were packaged under vacuum and stored in a conventional freezer (−20 °C) for a 40-day period. After frozen storage, the samples were thawed (4 °C) for 24 h and aged for the same durations used for the control treatment. In both treatments, the experimental units of each animal were randomly distributed with respect to aging time.

2.2. pH, exudate losses and water holding capacity

The aged beef samples were removed from the package, dabbed dry and weighed again to determine the purge, expressed as a percentage. The whole steak from each treatment was used to analyze cooking loss and tenderness using the Warner–Bratzler Square Shear Force method (WBsSF). The half steaks were used in the pH and water holding capacity (WHC) analyses. Samples were removed and frozen for later fragmentation index (FI) and sarcomere length analysis.

The pH was measured using a calibrated portable pH meter (Model HI 99163; Hanna, Woonsocket, RI, USA) with an insertion electrode, and samples were removed for determination of WHC; both of these measurements were conducted in triplicate.

The WHC analysis was carried out by the filter paper press method (FPPM) described by Honikel and Hamm (1994), with minor modifications. Samples of approximately 300 mg were placed onto a previously dried filter paper, and the assembly was pressed with a 5 kg-weight for 5 min. After pressing, the pressed meat area (PMA) and exudate liquid area (ELA) on the filter paper were obtained using the ImageJ® 1.42q software (National Institute of Health, USA), and the WHC was expressed as a PMA/ELA ratio.

For determination of the cooking loss, the whole steaks were weighed and grilled at 160–180 °C (Mega Grill; Britain, Curitiba, PR, Brazil) until they reached an internal temperature of 71 °C (AMSA, 1995), monitored by a digital thermometer (TD-880 with K-type thermocouple; ICEL, Manaus, AM, Brazil) inserted into their geometric centers. After cooking, the sample was cooled at room temperature for 2 h and weighed again. The cooking loss was determined by the difference in the weight of the steak before and after cooking, and the result was expressed in a percentage. The total exudate loss was determined as the sum of the purge and cooking loss.

2.3. Fragmentation index

The fragmentation index was determined by the protocol described by Davis, Dutson, Smith, and Carpenter (1980), with minor modifications. Five grams of sample, still frozen, were homogenized (Turratec TE 102; TECNAL, Piracicaba, SP, Brazil) in 50 mL refrigerated 2 mM KCl and 0.25 M sucrose solution (4 °C) at 15,000 RPM for 40 s. The homogenate was vacuum-filtered (Vacuum pump NOF-650, New Pump, Brazil) using a 250 μm-pore nylon screen that had been previously dried and weighed. The screen containing the residue was transferred to a filter paper that had been previously dried and weighed, and the screen and paper were weighed after 10 min at room temperature. The residue weight (RW) was determined by difference, and the FI

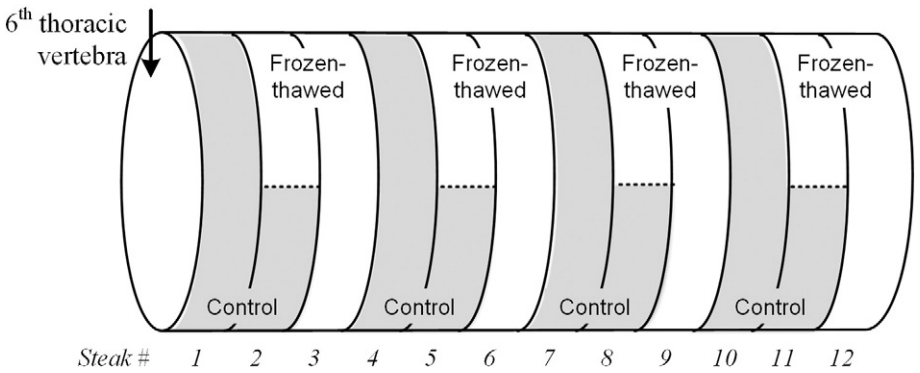

Fig. 1. Anatomical description of the division of *longissimus thoracis* steaks in the two treatments: Control = fresh samples aged at 1 °C; and Frozen–thawed = samples frozen (−20 °C) for 40 days, thawed (4 °C/24 h) and aged at 1 °C.