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Comparison of fresh beef and camel meat proteolysis during cold storage

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ABSTRACT

The objective of this research was to determine the difference in myofibrillar fragmentation of camel meat and beef during postmortem aging. Semitendinosus muscle was excised at slaughter and muscle pH was measured at 6, 12, 24, 48, and 72 h postmortem. Myofibril fragmentation index was measured on 1, 3, 5, and 7 days postmortem. Also, myofibrils isolated from semitendinosus muscles of camel and cattle at 1, 3, 5 and 7 days postmortem storage were analyzed using sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis. Results showed that the camel semitendinosus muscle had significantly higher myofibril degradation values compared to that in beef which was supported by a difference in troponin-T degradation and appearance of a 30 kDa band. Postmortem pH decline of camel meat was significantly slower than that of beef. This study demonstrated that the semitendinosus protease activity of camel meat was superior to that of beef, which may have been due to the difference in pH decline.

1. Introduction

Measurement of myofibril fragmentation is one of the most widely used methods to determine postmortem proteolysis in meat and has been widely used in studies on beef, pork, chicken and lamb (Olson, Parrish, & Stromer, 1976). Several different methods have been developed to measure the myofibril fragmentation in muscle. All the methods involve homogenization of the muscle followed by different techniques to determine the size of the resulting myofibril fragments. The first method developed to measure myofibril fragmentation examined the myofibrils by microscopy (Takahashi, Fukazawa, & Yasui, 1967). The myofibril fragmentation was calculated as the percentage of myofibrils that contained more than four sarcomeres, relative to the total number of myofibrils. Another method determined the size of the myofibril fragments by stirring the myofibrils through filters of specific pore sizes (Davis, Dutson, Smith, & Carpenter, 1980). The weight of the myofibrils remaining on the filters was then used to calculate the myofibril fragmentation. The most used method is based on the turbidity of the myofibrils adjusted to a common protein concentration (Davey & Gilbert, 1968). The turbidity is determined by measuring the absorption at 540 nm of the myofibril suspension. Myofibrillar fragmentation has been shown to be highly correlated with indices (shear force and sensory panel tenderness) of meat tenderness (Moller, Vestergaard, & Wismer-Pederson, 1973; Olson et al., 1976). Davey and Dickson (1969) demonstrated that turbidity of myofibril suspensions prepared by controlled homogenization increased with postmortem storage. Olson et al. (1976) used the protocol of Davey and Dickson (1969) but multiplied the turbidity values by a constant number and named it the myofibril fragmentation index (MFI). This index is a very useful indicator of meat tenderness, particularly for muscles not big enough to determine shear force or sensory tenderness (Veiseth, Shackelford, Wheeler, & Koohmaraie, 2001). Myofibril fragmentation accounts for more than 50% of the variation in tenderness of longissimus muscle (Culler, Parrish, Smith, & Cross, 1978). An increase in meat tenderness, which occurs during cold storage after rigor mortis, results from the degradation process in intra- and extra-cellular structures of muscle fibers (Nagaraj, Anilakumar, & Santhanam, 2005). Previous studies have shown that the accumulation of the 30-kDa component paralleled the changes in postmortem meat tenderness (Ouali, 1984). Farouk, Price, and Salih (1992) suggested that the appearance of 30-kDa components might be used as an index of only the rate of proteolysis, but not of tenderness. Ho, Stromer, and Robson (1994) further proposed that the degradation of troponin-T and the appearance of the 30-kDa component indicated not only postmortem proteolysis, but also the disintegration of the postmortem muscle Z-disk.

The world population of dromedary and bacterian camels is estimated to be 17 million (Elgasim & Alkanhal, 1992). Compared to other livestock, the camel is unique in having an exceptional ability to survive and thrive under adverse climatic conditions such as high ambient temperatures, low rainfall, and feed scarcity. Therefore, it offers an ideal option for animal production in arid and semi-arid regions of the world. The camel is a good source of meat in areas where the climate adversely affects other animal's production efficiency (Kadim et al., 2006). In spite of its potential,

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the contribution of camel meat to the per capita meat consumption in the world is not impressive (Elgasim & Alkanhal, 1992). Carcass characteristics of the camel are comparable to those of the other red meat animal species. The meat of young camels (below 3 years) is comparable in taste and texture to beef (Dawood, 1995). However, there remains the public perception that camel meat is tougher and of lower quality than beef causing camel meat consumption to be restricted to people in arid and semi-arid regions of the world.

The objective of the present study was to determine the possible difference in myofibrillar fragmentation between semitendinosus muscles of camel meat and beef in animals 3–5 years old.

2. Materials and methods

Semitendinosus (ST) muscles were obtained from 20 carcasses of bovine and camel of 3–5 years old 6 h after slaughter at room temperature. Each day five muscle samples were taken and stored at room temperature (25 °C) for 12 h and then stored at 4 °C for 7 days. The pH was measured at 6, 12, 24, 48 and 72 h postmortem, using a pH temperature probe (Eutech) inserted 2 cm each time into a section of the muscle. MFI was determined using a slightly modified version of the procedure of Culler et al. (1978). Samples were removed at 1, 3, 5 and 7 days postmortem at 4 °C and visible amounts of fat and connective tissue were removed. Four grams of minced muscle were homogenized for 30 s in 10 vol (v/w) of a 4 °C isolating medium consisting of 100 mM KCl, 20 mM potassium phosphate, 1 mM EDTA, 1 mM $MgCl_2$, and 1 mM sodium azide. The homogenate was sedimented at 1000*g* for 15 min and the supernatant decanted. The sediment was resuspended in 10 vol (v/w) of isolating medium using a stir rod, sedimented again at 1000*g* for 15 min and the supernatant decanted. The sediment was resuspended in 2.5 vol (v/w) of isolating medium and passed through a polyethylene strainer (18 mesh) to remove connective tissue and debris. An additional 2.5 vol (v/w) was used to facilitate passage of myofibrils through the strainer. Protein concentrations of the suspensions of myofibrils were determined by the biuret method of Gornall, Bardawill, and David (1949). Briefly, 0.25 ml of each suspension was placed into glass tubes and 0.75 ml MFI buffer was added, and finally, 4 ml biuret reagent was added and vortexed. Glass tubes were placed in the dark at room temperature for 30 min. The absorbance was read at 540 nm. Bovine serum albumin (BSA) was used to establish a standard curve. An aliquot of the myofibril suspension was diluted in isolating medium to a protein concentration of 0.5 ± 0.05 mg/ml. Protein concentration was again determined by the biuret method of Gornall et al. (1949). The diluted myofibril suspension was measured immediately at 540 nm. Absorbance was multiplied by 200 to give a MFI.

Myofibrils were isolated according as described by Olson et al. (1976). In brief, myofibrils were isolated by washing six times in 100 mM KCl, 20 mM K-phosphate (pH 7.0), 1 mM EDTA, and 1 mM sodium azide. Electrophoresis was conducted according to the procedure of Nagaraj et al. (2005). Results were analyzed using the SAS package (version 8). The least significant differences (LSD) test was used at the 5% significance level. All measurements were done in three replications.

3. Results and discussion

pH profile for camel and cattle ST muscle is shown in Fig. 1. The pH decline was slower for camel meat than beef, the pH of camel meat was 6.5 after 6 h postmortem, which was significantly higher than that of the cattle (6.1). However, all carcasses had a low ultimate pH of 5.5, with beef carcasses reaching this within 24 h postmortem, while camel carcasses reached their ultimate pH after

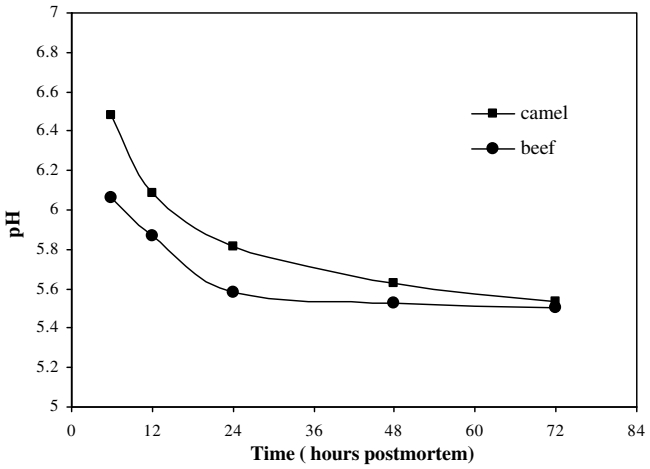

Fig. 1. pH decline for ST muscle of camel and beef.

48 h postmortem. The pH decline of meat is related to the glycogen content of the muscle at slaughter, where lower glycogen contents may result in decreased rates of glycolysis; hence, a slower accumulation of lactic acid and a slower rate of post-slaughter pH decline. Camels are gluconeogenesis animals due to its having humps. The amount of enzymes in its glycolytic pathway is, therefore, less than in cattle causing slower glycogen degradation and pH decline (Immonen & Puolanne, 2000).

MFI increased significantly ($p < 0.01$) in the ST muscles of both camel meat and beef from 1 to 7 days postmortem at 4 °C (Fig. 2). In addition, species significantly affected ($p < 0.01$) MFI. One possible cause of this variability could be the difference in the enzyme content and more likely in the enzyme/inhibitor ratio, a parameter reflecting the efficiency of the proteolytic systems. It has been found that the postmortem proteolytic process varies among species (Caballero et al., 2007). Because of this, the rate and extent of postmortem proteolysis of key myofibrillar proteins are a major source of variation in tenderness (Koohmaraie, 1996) and could be responsible for the differences in tenderization rates observed among different breeds or genotypes (Campo et al., 2000; Monson, Sanudo, & Sierra, 2004) .

Peptidase inhibitors play an essential role in meat tenderness, as is the case for calpastatin, a calpain inhibitor, cystatins, and a family of cysteine peptidase inhibitors (Ouali & Talmant, 1990). Cheret, Delbarre-Ladrat, Lamballerie-Anton, and Verrez-Bagnis (2007) found that the calpain content was similar in both fish and bovine muscles but calpastatin levels were different in the muscles studied. Ouali and Talmant (1990) also reported that muscle calpastatin

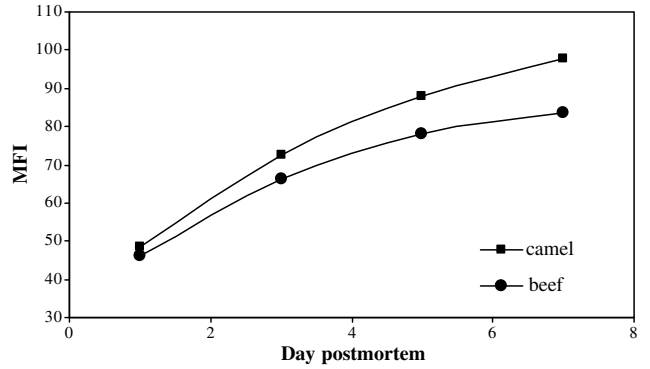

Fig. 2. Effect of postmortem storage time on the fragmentation index of myofibrils from eight camel and bovine ST muscles during postmortem storage at 4 °C.

levels are not the same among mammalian families. Factors that may contribute to tenderization during the first 48 h post-slaughter include pH decline (glycolysis), temperature and their effect on muscle protease activity and muscle shortening. Reports have stated that an intermediate pH decline is optimal for calpain activity; i.e., pH 6.0 at 1.5 h postmortem as suggested by Hwang and Thompson (2001). Claeys, De Smet, Demeyer, Geers, and Buys (2001), in a study on pork, showed there was a significant correlation between activities of several enzymes and pH values at 1 h postmortem, suggesting that faster postmortem pH decline results in lower calpain activities due to more pronounced denaturation. Maddock, Huff-Lonergan, Rowe, and Lonergan (2005) found that calpain activity was highest at pH 6.5, and that inhibition of μ-calpain by calpastatin was not affected by pH, allowing for proteolytic activity of μ-calpain with a slower rate of autolysis (self-proteolysis and inactivation by calpastatin). A more rapid pH decline may inactivate μ-calpain, causing a reduction in the proteolysis of myofibrillar proteins and subsequent postmortem tenderization. Marsh, Ringkob, Russell, Swarts, and Pagel (1987) reported that as pH values at 3 h postmortem decrease to below 6.1, meat tenderness gets lower, possibly since a decrease in this value is associated with a shorter period of the time when important proteases are active. In the present study, the pH decline was faster in beef than in camel meat during the first 24 h postmortem. Therefore, there is a possibility that the higher MFI observed in camel meat compared to beef was due to the higher pH of camel meat during the first 48 h postmortem, meaning more optimal conditions for higher protease activity and, thus, increased proteolysis.

SDS 13% polyacrilamide gels of camel and bovine ST muscle myofibrils prepared from muscles after 1, 3, 5 and 7 days postmortem storage at 4 °C showed that nearly identical changes occurred in the myofibrillar proteins in both meats (Fig. 3), that is, troponin-T band gradually became less intense and disappeared concurrently as a 30-kDa band appeared during postmortem storage. No other major changes occurred in the other myofibrillar proteins. These results imply a degree of proteolysis in the muscle as indicated by the appearance of a 30-kDa band. It has been confirmed that the 30-kDa band is a proteolytic product of troponin-T (Ho et al., 1994). In beef, the 30-kDa component was absent up to 3 days of storage and then started to appear. The appearance of a 30-kDa band at 3 days of storage in camel meat also implied that this meat had a higher degree of proteolysis. The major contractile proteins, myosin and actin, were changed little during aging. Koohmaraie, Seideman, Schollmeyer, Dutson, and Babiker (1988) reported similar results during cold storage. Bandman and Zdanis (1988) and Yates, Dutson, Caldwell, and Carpenter (1983) also observed the same events where myosin and actin did not appear to undergo degradation during postmortem storage at 4 °C.

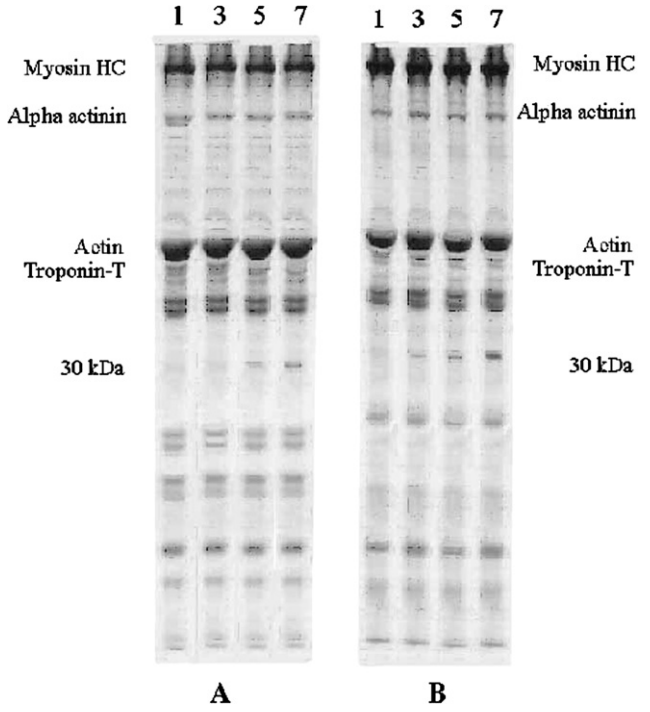

Fig. 3. SDS–PAGE (13%) of myofibrils isolated from (A) beef ST muscle and (B) camel ST 12 muscle after 1, 3, 5 and 7 days postmortem storage at 4 °C.

4. Conclusion

The present study provides evidence that the protease activity in the ST of camel meat is significantly higher than that of beef. Camel meat had a slower pH decline than beef, which may be due to a lower glycogen content likely to result in a decreased rate of glycolysis and, consequently, to a slower accumulation of lactic acid and to a slower rate of post-slaughter pH decline. This may be linked to increased protease activity of camel meat. In the proteolytic process, the Z-line degrades, which results in fragmentation of myofibrils and in the appearance of 30-kDa components. Further investigation is needed into early postmortem muscle protease activity in camel meat and beef to explain the difference in myofibril fragmentation.

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