PE1.25 Factors affecting tenderness in bovine longissimus dorsi, a multivariate approach 198.00

Kristin Hollung (1) kristin.hollung@nofima.no, Eva Veiseth-Kent(1), Laila Aass (2), Kjell Ivar Hildrum (1)
(1)Nofima Mat AS, Norway
(2)University of Life Science, Norway

Abstract— Tenderness is a critical factor determining the consumer's acceptance of meat. It is now well established that meat tenderisation is connected to the calpain-mediated proteolysis of key myofibrillar and associated proteins. In this study samples from 403 Norwegian Red (NRF) dual purpose bulls were collected over a 4 year period and measured for pH, tenderness, color and protease activity. By using a multivariate approach we have shown that tenderness (WB Shear Force) is correlated with pH at different post mortem time points. It is also shown that tenderness is correlated both with the activity of calpastatin and with the ratio between µ- or m-calpains and calpastatin.

K. Hollung, E. Veiseth-Kent and K.I. Hildrum is at Nofima Mat, Osloveien 1, N-1430 Ås, Norway (corresponding author K. Hollung phone: +47 64 97 01 42; fax: +47 64 97 03 33; e-mail: kristin.hollung@nofima.no, eva.veiseth-kent@nofima.no, kjell.ivar.hildrum@nofima.no). L. Aass is at Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O.Box 5003, N-1432 Ås, Norway (e-mail: laila.aass@umb.no).

Index Terms—tenderness, longissimus dorsi, pH.

I. INTRODUCTION

Tenderness is a critical factor determining the consumer's acceptance of meat. However, significant variations in the tenderness of beef can be found at the retail and food service levels, and the unpredictable nature of this variation can have serious economic impacts on the meat industry. It is well known that meat tenderness improves during post mortem storage, and that this tenderisation process is connected to the calpain-mediated proteolysis of key myofibrillar and associated proteins (for reviews, see Goll et al., 1991; Koohmaraie, 1996). The aim of the present work was to investigate the relationship between tenderness in bovine M. Longissimus dorsi (LD) and measurements of muscle properties shortly after slaughter in a large population of young bulls.

II. MATERIALS AND METHODS

A. Animals and sampling

A total of 403 Norwegian Red dual-purpose young bulls (approximately 13 months of age/450 kg live weight) kept at a performance test station (GENO-Breeding and AI Association) was included in this study. During the test period (150-330 days of age), the bulls were kept in pens with 15-23 bulls/pen. The bulls were slaughtered at a commercial abattoir in groups of 20-30 animals at different times of the year during the period from 2003-2006. Most carcasses were electrically stimulated (90 V) approximately 30 min post mortem, but 54 of the 403 carcasses were not subjected to electrical stimulation. The LD muscles were hot-boned from the carcass at 1 h post mortem, vacuum packed and kept at 12°C for the first 10 h to avoid cold-shortening. The vacuum packed muscles were then kept at 4°C for the rest of the storage period for later WBSF measurements. To monitor the conditioning temperatures, temperature loggers (EBI-125 A, Temperature Data Logger, Ebro Electronic, Ingolstadt, Germany) were inserted in some of the muscles and placed in the coolers throughout the storage period. Measurements of muscle pH were performed at 1, 3, 6, 10 and 48 h post mortem by inserting a glass-stick probe (InLab®427 Combination pH Puncture Electrode, Mettler Toledo Intl. Inc., Greifensee, Switzerland), connected to a pH meter (Portamess 752 Calimatic, Knick, Berlin, Germany) into the muscle.

B. Warner Bratzler Shear Force (WBSF) Measurements

WBSF were performed at 7 days post mortem. From each muscle, a 3.5 cm thick slice was vacuum-packed in polyethylene bags, heated in a water bath at 70.5ºC for 50 min, and then chilled in iced water for 50 min. The meat slices were cut into rectangular pieces of 1×1×3 cm along the fibre direction, and ten pieces from each sample were sheared perpendicular to the fibre direction with a triangular WBSF device attached to an Instron Materials Testing Machine (Model 4202, Instron Engineering Corporation, High Wycombe, UK).

The average maximum force (given as N/cm2) for the ten parallels from each sample was used in the data analysis.

C. Calpain and calpastatin activity

Samples that had been snap frozen approximately 1h post mortem were used for determination of μ- and m-calpain activity by casein zymography (Raser et al. 1995). Samples for calpastatin were extracted between 24 and 31 hrs post mortem using the heated calpastatin procedure of Shackelford et al. (1994). Following extraction and heat treatment, calpastatin activity was determined using BODIPY-FL labelled casein according to Thompson et al. (2000).

D. Measurements of color,

IMF and water Color (L*a*b) was measured by a MINOLTA CR-200 8 d post mortem at three positions (medial, intermediate and lateral; three replicates in each position) on the fresh surface of a 2.5 cm sliced muscle sample from each animal. The mean of the L, a and b values are presented here, only. After removing the epimysium and external fat, the slice was homogenised and stored at -20 ºC until analysis (Soxlet) of intramuscular fat (IMF). The water % was derived from the same lab procedure. E. Statistical analysis Initially, the data were adjusted for fixed effects using the GLM procedure in SAS (SAS, 1999). All dependent variables except μ- and m-calpain were adjusted for the effect of age and slaughter group x pen, μ- and m-calpain were additionally adjusted for day of analysis. The multivariate analysis was performed in the software package Unscrambler version 9.8 (CAMO A/S, Oslo, Norway). Partal least squares regression (PLSR) visualises the main variations in the data through principal components (PCs). Using PLSR, PCs were calculated and used to construct a coordinate system in such a way that PC1 is the one explaining the most variation in the data and PC2 the second largest variation and so on. The resulting score plot allows for easy interpretation of the main variation in the data set.

III. RESULTS AND DISCUSSION

We have collected samples from 403 Norwegian Red dual purpose bulls from 2003-2006 for measurements of pH, tenderness, color and protease activity, see Table 1. Among these animals we had no stress or DFD problems as can be seen from the pH measurements after 48 h, with all values below 5.8. A fraction of the carcasses (n=54) were not subjected to low-voltage electrical stimulation. As can be seen for the WBSF values both very tender and very tough muscles were observed regardless of electrical stimulation. Using a multivariate approach for analysis of the measurements we could observe some interesting features in the data. Partial least squares regression (PLSR) using the different measurements as the X-matrix and electrical stimulation as the Y-matrix showed that as much of 49% of the variation in the experimental results (X-matrix) could be explained by electrical stimulation in the first two principal components (Figure 1). As can be seen in the plot electrical stimulation is negatively correlated to pH after 1, 6, and 10 h as well as to calpastatin activity, while a positive correlation can be observed with the ratio between μ-calpain/calpastatin and m-calpain/calpastatin. Electrical stimulation had no influence on the ultimate pH 48 and color, measured as L*, a* and b* values. Thus, the electrical stimulation method used in the present study led to a decrease in early pH values and calpastatin activity post mortem, while the tenderization activity was positively affected. Using the same multivariate approach on tenderness, we did a PLSR with the different measurements as the X-matrix and WBSF as the Y-matrix. A plot of the first two principle components is shown in Figure 2. This shows that 31% of the variation in the data can be explained by WBSF. In the analysis it appears that WBSF was positively correlated to ultimate pH 48 and calpastatin activity and also to pH at 1, 6 and 10 h. On the contrary, WBSF was negatively correlated to the ratio of μ-calpain/calpastatin and m-calpain/calpastatin. All results demonstrate the beneficial effect of the calpain enzyme system on tenderness. Interestingly, however, there was less effect of the m- and μ-calpain activities per se on WBSF than the relationships shown above.

IV. CONCLUSION

Tenderness of bovine LD is influenced by many factors. Using a multivariate approach we have shown that tenderness (WBSF) is correlated with pH measured at 1, 6, 10 and 48 h post mortem. It is also evident that tenderness is correlated to the activity of calpastatin and to the ratio between μ- or m-calpain and calpastatin.

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