51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

PROTEOMIC ANALYSIS OF POST MORTEM CHANGES IN BOVINE MUSCLE

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Key Words: Bovine/ Muscle proteome/ Post mortem/ Meat quality

Introduction

Consumers rate tenderness as the major quality trait of beef [1], thus understanding factors involved in tenderness development is a major concern for the beef producing industry. Meat tenderness is a complex trait which is closely related to the biological traits of the live animal, hence biological sciences, including genetics, physiology, cell biology and biochemistry has been widely employed for decades to characterize the biological mechanism behind major variability of meat tenderness [2]. Especially, the post mortem glycolysis and proteolysis in muscle tissues have a major influence on tenderness and quality of meat [3, 4]. Proteolytic enzymes, including: calpains, cathepsins and proteasomes [5] have been proposed to participate in the post mortem protein degradation,. However, the biochemical mechanisms of the meat tenderization process are not yet well described.

2-DE based proteome analysis is a potent tool for characterizing post mortem changes in muscles, and recent studies of the post mortem proteomes of porcine [6-8] and fish [9] muscles have been used to identify altered patterns of myofibril proteins that occur during storage of meat. In particular, a number of metabolic proteins were found to change during post mortem storage of porcine meat [7]. Although the in vivo roles of these metabolic proteins are well known, their influence on post mortem changes, and the relations to meat tenderness remains unclear.

Objectives

The objective of the present work was to characterize proteome patterns of post mortem bovine muscle, with special attention to the changes in metabolic proteins during early post mortem storage of beef carcasses. The post mortem protein patterns of *M. Longissimus dorsi* (LD) and red part of *M. semitendinosus* (ST) were chosen for this study because the metabolic phenotypes of these two muscles are well known to differ.

Methodology

The experiment included 12 *Holstein Friesian* bull calves. The calves were slaughtered at the experimental house and samples were removed 15 min post mortem and 24 h post mortem from the red part of the ST and the LD. Soluble fraction of muscles was extracted in TES buffer by using ultraturrax. The running of two-dimensional gel electrophoresis (2DE) was performed according to the method described previously [6]. The second dimension was run vertically and the proteins were separated in 12% SDS-PAGE gels. Analytical 2DE gels were silver stained as previously decribed [6], while preparative gels for mass spectrometry (MS) were silver stained according to Shevchenko [10]. The 2DE gels were analysed using the ImageMaster^{TM} 2D Platinum software Version 5.0 (Amersham Biosciences). The spots were automatically matched with the spots of a reference gel. The spot report was imported into Unscrambler version 9.0 (CAMO A/S, Norway) and 50-50 MANOVA [11] (http://www.matforsk.no/ola/ffmanova.htm) for statistical analyses. An Ultraflex MALDI-TOF/TOF mass spectrometer with LIFT module (Bruker Daltonics) was used for protein identification.

Results & Discussion

Fig. 1 shows representative 2DE patterns of the TES-soluble protein fractions extracted from LD and ST muscles. The proteins in the molecular mass region of 10 kDa to 75 KDa, and the pH range between 4 and 7, were included in comparative analyses. Image analyses allowed matching and relative quantitation of 923 spots from the LD data set and 630 spots from the ST data set. The complete data set of spot intensities from comparative image analyses was analysed by PCA, and resulting score plots are presented in Fig. 2. The PCA score plots for LD and ST reveal that two clusters related to sampling time, i.e. at slaughter (T0) versus 24 h post mortem are formed. The results (data not shown) from rotation tests [11] indicate that 13 spots form the LD data set and 18 spots form the ST data set were significantly changed ($p<0.05$), and these spots were subjected to identification by MS. Among the identified spots, 5 were observed to change in similar patterns both in LD and ST muscles, namely cofilin, lactoyglutathion lyse, substrate protein of mitochondiral ATP-dependent proteinase SP-22, HSP27 and HSP20, while altered expression patterns of 15 spots were unique for either LD or ST (data not shown).

Expression patterns from comparative 2DE analyses contain complex information that is partly hidden if only classic statistical methods are used; hence increasing awareness of the advantage and usefulness of multivariate statistical methods has emerged [12]. In this study we have used PCA and rotation testing to explore the variation in the comparative 2DE patterns (Fig. 2). Furthermore, traditional significance testing for marker selection is not adequate for analysis of many variables, as the false discovery rates will be unacceptably high, as is often seen in the analysis of microarray and proteome data [13]. For example, 50 significant markers will always be selected by a 5% significant level from 1000 spots when using a traditional t-test [11]. We have therefore used a rotation test for adjusting the p-values, and have thereby reduced the false discovery rate.

The observed decreases in cofilin, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP27 and HSP20 as seen in both in LD and ST muscles indicate that these changes are immediate post mortem happenings, and has also been observed to occur in porcine muscles, that have much faster rigor development than cattle muscle. A metabolic enzyme, lactoylglutathione lyase, was increased in both muscles during early post mortem storage. This enzyme catalyses the first step of the glyoxal pathway, in which lactic acid is the end product [14]. Several altered spots were identified only in LD muscle. This may be due to different solubilisation properties of myofibril fragments of the two different muscles, and may be related to the rate of the tenderisation process in these muscles. Several subunits of 20S proteasomes and 26S proteasomes were identified to decrease in intensity post mortem in ST muscle. Several authors have previously suggested that proteasome complexes play a role in post mortem proteolysis and meat quality [5].

Biochemical and ultrastructural differences between the various fibre types in the two muscles may explain the differences observed in the protein pattern. These differences reflect distinct metabolic and physiological functions of the different muscles [15]. The energy metabolism, which is responsible for the rate and extent of the pH decrease post mortem, occurs at different rates in different types of muscles (red and white) [16]. Several studies have shown that the metabolic properties of muscles are related to variation in glycogen content and ultimate pH of meat [17, 18].

Conclusions

In this study, we have compared the protein patterns of two different bovine muscles (LD and ST) at two time-points post slaughter, in order to study changes in their post mortem proteomes. Five proteins, namely cofilin, lactoylglutathion lyse, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP27 and HSP20, were changed in both ST and LD muscles during post mortem storage. Fifteen additional protein changes were observed in either ST or LD muscles. Further study is required to reveal how the reported changes are related to meat quality traits of cattle.

References

[1] Grunert, K. G., Food. Qual. Prefer. 1997, 8, 157–174.

[2] Koohmaraie, M., Meat Sci. 1996, 43, S193–S201.

[3] Marsh, B. B., Ringkob, T. P., Russell, R. L., Swartz, D. R. and Pagel, L. A., Meat Sci. 1987, 21, 241–248.

[4] Ohalloran, G. R., Troy, D. J. and Buckley, D. J., Meat Sci. 1997, 45, 239–251.

[5] Sentandreu, M. A., Coulis, G. and Ouali, A., Trends Food Sci. Technol. 2002, 13, 400–421.

[6] Lametsch, R. and Bendixen, E., J. Agric. Food Chem. 2001, 49, 4531–4537.

[7] Lametsch, R., Roepstorff, P. and Bendixen, E., J. Agric. Food Chem. 2002, 50, 5508–5512.

[8] Lametsch, R., Karlsson, A., Rosenvold, K., Andersen, H. J., et al., J. Agric. Food Chem. 2003, 51, 6992–6997.

[9] Kjaersgard, I. V. H. and Jessen, F., J. Agric. Food Chem. 2003, 51, 3985–3991.

[10] Shevchenko, A., Wilm, M., Vorm, O. and Mann, M., Anal. Chem. 1996, 68, 850–858.

[11] Langsrud, O., Stat. Comput. 2005, 15, 53–60.

[12] Jessen, F., Lametsch, R., Bendixen, E., Kjaersgard, I. V. H. and Jorgensen, B. M., Proteomics 2002, 2, 32–35.

[13] Dudoit, S., Shaffer, J. P. and Boldrick, J. C., Stat. Sci. 2003, 18, 71–103.

[14] Kim, N. S., Umezawa, Y., Ohmura, S. and Kato, S., J. Biol. Chem. 1993, 268, 11217–11221.

[15] Pette, D. and Staron, R. S., CIBA Found. Symp. 1988, 138, 22–34.

[16] Whipple, G., Koohmaraie, M., Dikeman, M. E., Crouse, J. D., et al., J. Anim. Sci. 1990, 68, 2716–2728.

[17] Henckel, P., Oksbjerg, N., Erlandsen, E., Barton–Gade, P. and Bejerholm, C., Meat Sci. 1997, 47, 311–321.

[18] Henckel, P., Karlsson, A., Jensen, M. T., Oksbjerg, N. and Petersen, J. S., Meat Sci. 2002, 62, 145–155.

Tables and Figures

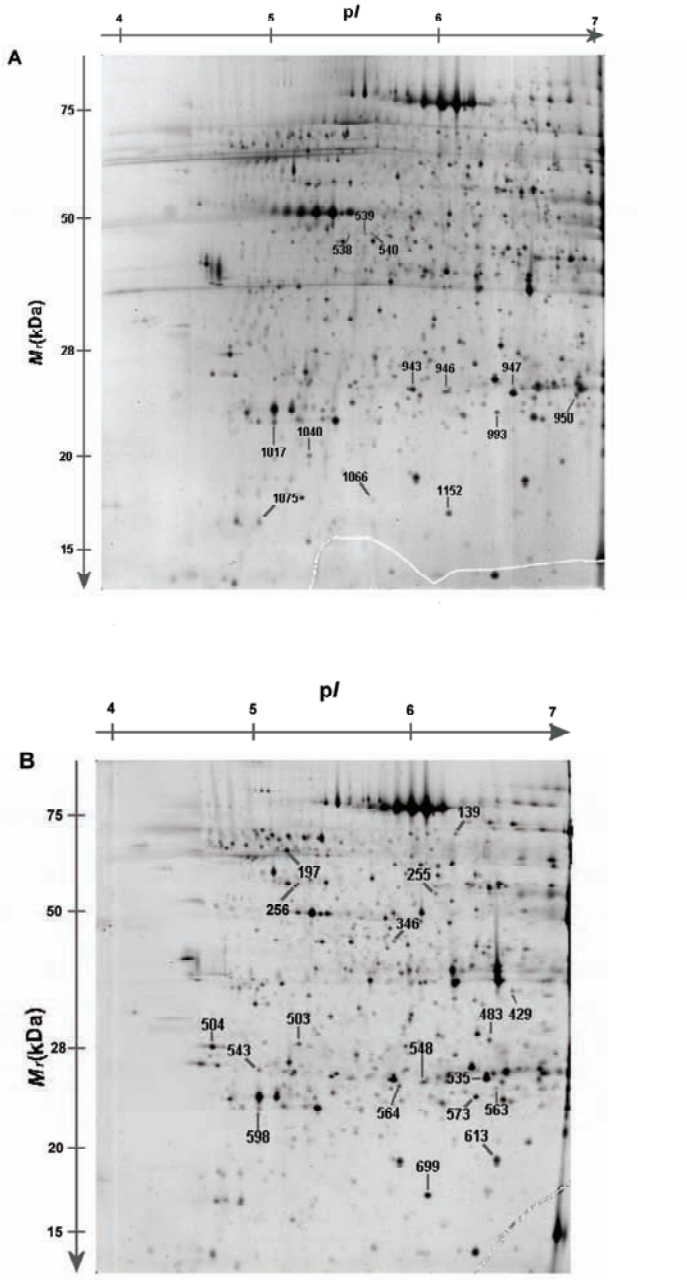
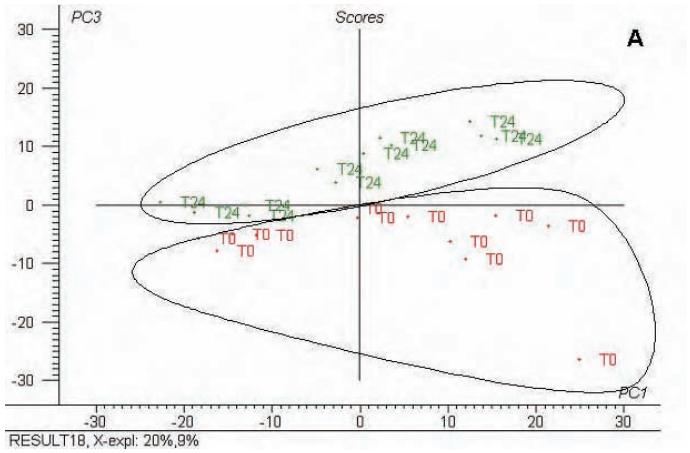


Figure 1. Silver-stained 2DE master gel of A) bovine *M. longissimus dorsi* (LD) muscles and B) bovine *M. Semitendinosus* (ST) muscles. Marked spots were identified by mass spectrometry.



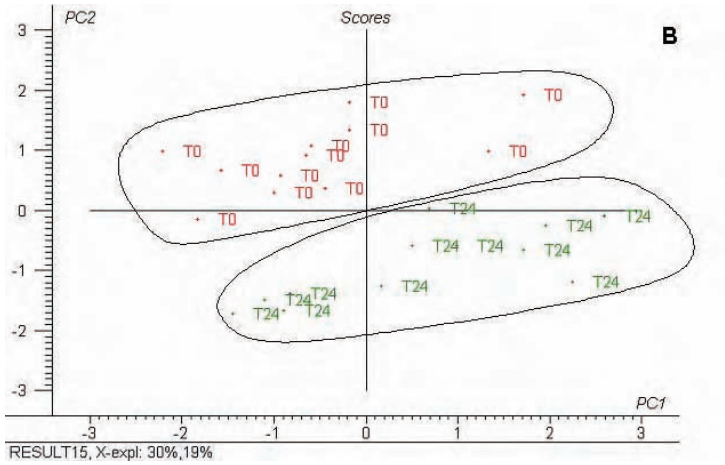


Figure 2. Spot intensities from comparative image analysis were analysed by principal component analysis (PCA). A) PCA score plot of 923 matching spots from bovine *M. longissimus dorsi* (LD) muscles related to post mortem storage time. B) PCA score plot of 630 matching spots from bovine *M. Semitendinosus* (ST) muscles related to post mortem storage time. T0: samples taken at slaughter time, T24: samples removed from the carcass 24 h after slaughter. Circles are drawn to show the two clusters relating to sampling time.