PE1.24 Proteolysis and meat quality in different bovine breeds. 197.00

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Abstract Meat maturation process is known to be related to proteolysis occurring postmortem but the exact mechanism governing protein degradation is not yet completely understood. In this study we compared maturation process between Longissimus thoracis (LT) and Biceps femoris (BF) for four different bovine breeds: Angus (AN), Herens (HR) Limousin (LI) and a second generation hybrid having 75% LI genotype and 25% Red Holstein genotype (F2). Calpain activity and protein degradation revealed the existence of at least two proteolysis mechanisms allowing separation of proteins based on their different degradation rates. For this study, two degradation rates were selected, first group, named ¡§fast degrading proteins¡¨, which members are more than 50% degraded at 48 hours postmortem and ¡§slow degrading proteins¡¨, which partial degradation appeared only 14 days postmortem. The muscles of the HR breed showed the slowest efficiency of degradation as compared to the other breeds but it did not influence meat tenderization. On the contrary, in muscles from the LI breed proteolysis processes were the most effective of the four breeds and this observation correlated with a better tenderization of the meat.

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Index Terms: Meat tenderness, proteolysis, bovine breeds.

I. INTRODUCTION

There is a large body of literature on investigations into associations of biochemical traits with variations in meat tenderness [1]. The major aim of this work is to identify better biochemical markers to use as predictors of meat tenderness. It assumed that one of the key players in the proteolysis process is mu-calpain, a calcium-dependent neutral peptidase present in the skeletal muscle [2]. Whereas considerable evidences now suggest that another calpain: m-calpain, also present in the skeletal muscle, does not participate to myofibrillar proteins degradation during refrigeration storage [3]. Only few studies look at the correlation between the breeds and the meat quality in cattle, the majority of the genetic works being dedicated to find genetic markers of meat tenderness. Here we focused on the comparison of four distinct bovine breeds. Through out biochemical analysis, we compared meat maturation process and correlated these data with effective meat tenderness, a measured determining the Warner Bratzler Shear Force.

II. MATERIALS AND METHODS

A. Animals

A total of 32 animals were considered in this study. They belonged to four different breeds: Angus (AN), Herens (HR; a typical Swiss breed), Limousin (LI) and a second generation hybrid having 75% genotype LI and 25% Red Holstein. The animal were slaughtered at a weight of less then 280Kg. Meat was matured at 4¢XC under vacuum for the indicated times.

B. Total protein extraction and analysis

Samples from the LT and BF muscles were collected at 1 hour, 48 hours and 14 days postmortem. Total protein extracts were prepared in lysis buffer (50 mmol/l Sodium Phosphate, 2% Sodium Dodecyl Sulfate) supplemented with 10ƒÝmol/l of Phenyl Methyl Sulfonyl Fluoride and 10ƒnƒÝmol/ of E64. Protein were then separated on denaturing SDS-PAGE gels and analyzed directly for titin and nebulin contents or by western blotting using appropriated antibodies for vinculin, meta-vinculin and desmin. All the primary and secondary antibodies were purchased from Sigma. Chemiluminescence was performed using LiteAblot (Euroclone) and revealed using GeneGnome (Syngene). The % of degradation was calculated as follow: (Band intensity t= 48hours or 14 day- band intensity t= 1hour)/ Band intensity t=1hour *100.

C. In gel zymography

Activity of mu- and m-calpain were determined in sarcoplasmic fractions of LT and BF samples collected at 1 hour, 48 hours and 14 days postmortem using casein zymography. The

sarcoplasmic protein extraction procedure and the casein zymography technique used were described previously by [4].

D. Warner Bratzler Shear Force Determination

Muscle samples were vacuum-packaged and stored at -20°C until Warner Bratzler Shear Force determination. Frozen samples were thawed at 2°C for 24 hours and slices were held at room temperature for 1 hour before cooking. Shear force was determined on the cooked sample cooled to ambient temperature; ten 1.27-cm-diameter cores were obtained from each slice and sheared perpendicular to muscle fibers orientation using a Texture Analyzer TA HDi device (Stable Micro Systems) and maximum shear force recorded.

E. Statistical Analysis

Anova test was used to analyze variance between groups. When possible, Newman-Keuls test was performed. Values of $p<0.05$ were considered significant.

III. RESULTS AND DISCUSSION

We first analyzed pH decrease postmortem in all breeds considered in this study. No differences were observed in the ultimate pH (t=24 hours). Nevertheless, in LT muscle the pH in HR breed was higher at 3 and 5 hours postmortem while compared to other breeds, suggesting a lower decrease kinetic. The same observation were recorded for the BF muscle, in HR and AN breeds, however, in these cases the differences were less marked and concerned. In order to compare meat maturation process in the different bovine breeds, we first analyzed the activity of *ƒÝ*- and m- calpains by in gel zymography. Only mu-calpain showed a decrease in activity over time, whereas for m-calpain no decrease in activity was observed. The decrease of the activity of mu-calpain postmortem observed in gel zymography, is assumed to result from an autolysis mechanism [4]. Therefore, such decrease can be used to evaluate its in vivo activity. Activity of mu-calpain decreased quite rapidly and about 80% of degradation was already reached 48 hours postmortem in LT muscle of AN and LI breeds (AN: 83% and LI: 74%). A lower rate of mu-calpain autolysis was also observed in F2 and HR breeds but to a lesser extent (F2: 53% and HR: 47%) (figure 1). In the BF muscle, only HR breed showed a less important degradation of mu-calpain 48 hours post mortem, whereas in LI breed, almost all the m-calpain was degraded at this time. Finally, in both LT and BF muscles, no residualmu-calpain activity was revealed at 14 days postmortem. On the contrary, m-calpain activity did not decrease in both LT and BF muscles of any breeds, suggesting that this protease was not active and not degraded. We then monitored the degradation of several proteins considered as hallmarks of meat maturation or tenderization. The list of proteins analyzed included vinculine, meta-vinculin, titin, nebulin and desmin. The proteins analyzed could be divided into two sub-groups: the first composed of meta-vinculin, titin, and nebulin, included proteins, which degradation postmortem was relatively rapid and reached a degradation rate of about 50% 48 hours postmortem. This observation is well represented in figure 2, which reports degradation rates of meta-vinculin (a fast degrading protein) and vinculin (a slow degrading protein) in LT muscle 48 hours postmortem. Such degradation kinetics was compatible with the involvement of mu-calpain as the major protease responsible for the degradation of these proteins. A second group, including desmin and vinculin, showed a much slower degradation. At 48 hours postmortem it was almost impossible to observe any degradation, which became visible only at 14 days postmortem. However, the degradation rate remained lower than 60%. These data confirm that proteolysis, after slaughtering, is effective all along the meat maturation process, with a first important step occurring in the first day postmortem and involving mu-calpain as the major protease. When comparing protein degradation in the different considered breeds, it appeared clear that meat obtained from the HR breed maturated slower as compared to meat obtained from the others. On the contrary, meat obtained from LI breed had the faster protein-degradation rates for almost all the proteins analyzed. This suggested that the meat of HR breed, maturated for 14 days after slaughtering, would be the less tender, while the meat of LI breed would be the more tender as compared to all. This hypothesis was partially confirmed by Warner Bratzler Shear Force determination (figure 3). Indeed, this analysis showed that meat of LI breed was the more tender of the group, while no differences between other breeds were noticed. The fact that meat obtained from HR breed was not tougher may be due to the fact that proteins, with a fast degradation rate, are completely degraded at 14 days postmortem even in HR breed. Moreover, this lack of difference also

suggests that the lower degradation of slow degrading proteins is not sufficient to influence the meat tenderization process.

IV. CONCLUSION

The aim of this study was to compare the maturation process of LT and BF muscles in different bovine breeds. Calpains activity, degradation rate and Warner Bratzler shear force were determined. The main finding of this work was the revelation of potential several proteolysis mechanisms involved in meat maturation. These mechanisms act on structural proteins with different kinetics allowing to defining markers of meat maturation into at least two subgroups: “slow” and “fast” degrading proteins. Degradation of ¡§fast degrading proteins is suggested to involve mu-calpain activity. We also observed differences in the degradation rate between species. These differences did not influence meat tenderness, with the exception of meat from the LI species, which was the more tender of the groups considered. The meat obtained from HR breed did not result tougher as compared to the others. This observation is probably due to the long time of meat maturation (14 days) before determining Warner Bratzler Shear Force. It would be interesting to determine tenderness earlier during the maturation process.

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LEGENDS TO THE FIGURES Figure 1: % of degradation of mu-calpain in LT muscle at 48 hours postmortem Figure 2: Degradation rate of Meta-Vinculin (A) and Vinculin (B) in LT muscle 48 hours postmortem Figure 3: Warner Bratzler Shear Force determination in LT (A) and BF (B) muscles.