Prediction equations of beef tenderness: implication of oxidative stress and apoptosis

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Abstract— **Over the last years, several studies have generated a list of protein potential markers of beef tenderness by comparing extreme groups of tenderness, using two-dimensional electrophoresis and mass spectrometry. In the present work, we have developed prediction equations of mechanical shear-force measures from the abundances of 24 of these protein markers for *Longissimus Thoracis* (LT) and *Semitendinosus* (ST) muscles. The samples provided from Charolais young bulls and steers of the French MUGENE program (Genanimal-APISGENE). Protein abundances were quantified by Dot-Blot, an immunological technique, using specific antibodies for each protein. Shear-force measures of the 222 samples were obtained with the Warner-Bratzler device. On both muscles, the tenderness prediction equations were obtained by multiple regression analysis of shear-force measures on protein abundances. The LT-muscle equation predicts toughness measures with an error of 5.7 % on 56 % of samples. Major explicative proteins are Heat Shock Protein 20 ($P < 0.0001$), Heat Shock Protein 70-GRP75 ($P < 0.05$), αB-Crystallin ($P < 0.05$) and cis-peroxiredoxin-6 ($P = 0.001$). The ST-muscle equation predicts toughness measures with an error of 5.9 % on 57 % of samples. The most explicative proteins are cis-peroxiredoxin-6 ($P < 0.0001$), Heat Shock Protein 40 ($P < 0.05$), Heat Shock Protein 70-GRP75 ($P < 0.05$) and Heat Shock Protein 70-1A ($P = 0.001$). These proteins are involved in oxidative stress resistance, by anti-oxidant activity (such as cis-peroxiredoxin) or chaperoning activity (such as Heat Shock Proteins). This study demonstrates the importance of apoptosis and oxidative stress on meat tenderness predictability.**

Keywords— **tenderness, beef, markers**

I. INTRODUCTION

Beef tenderness is an important question for consumers. It shows a high variability unchecked by the industry which requests predictive test of this quality. In order to develop relevant tests in France, various research programs have been conducted in collaboration between scientists and the beef industry for several years. These functional genomics programs have identified potential markers of tenderness (Bernard et al., 2007, Guillemin et al. 2009a), at DNA, mRNA and proteins levels, in different contexts of production (breeds, muscles, types of animals). An immunological test (dot-blot) has been developed to quantify the abundance of protein markers. The objective is to apply this type of test for the evaluation of tenderness potential of a live animal or of meat. To develop such test, equations for predicting the tenderness from the abundance of protein markers, must first be developed. The aim of the present study was to develop this tool, for predicting the tenderness of *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles. For this we analyzed the relationship between tenderness and the abundances of 24 potential protein markers of tenderness, and established prediction equations.

II. METHODS

We used animals from the French Mugene program (ANR-Genanimal APISGENE) (Hocquette et al., 2008), including 67 young bulls and 44 steers from Charolais breed. The young bulls were fattened in intensive system and steers were castrated at 3 months and fed on pasture. All the animals were slaughtered at the abattoir of INRA Clermont-Ferrand/Theix at 17 months of age on average for young bulls and 30 months for steers. Samples of LT (n = 111) and ST (n = 111), were collected within 10 minutes after slaughter, frozen in liquid nitrogen and stored at -80 °C until analysis. Steaks from each muscle were aged at 4°C for 14 days and then frozen at -20°C. After thawing, they were grilled to a core temperature of 55°C, and the shear force of each meat sample was measured using Warner-Bratzler (WB) test. From muscle samples, the extracted proteins were quantified by dot-blot using the

technique described by Guillemin et al. (2009b). Briefly, primary antibodies specific for each of the 24 proteins were hybridized and revealed by fluorescent secondary antibodies, from 15 micrograms of total protein from each sample. The quantifications were performed according to a range of standards. All the available data (measurements of protein abundances and WB) have been fixed and centered to account for the effect of slaughter year (2003 or 2005). To calculate a prediction equation, linear regressions were performed with the lm function of R (version 2.12.1) using the least square method. In this regression, the WB score corrected for the year was the dependent variable to be explained, and the abundances of 24 proteins, the quantitative explanatory variables. An equation was established for each muscle. In order to assess the predictability of the equation for each sample, the absolute value of the difference between the predicted value of WB (Vp) in the equation and that measured (Vm) was calculated, relative to the measured value, and percentage (|Vp-Vm|/Vm x 100). This constitutes the error of the prediction.

III. RESULTS AND DISCUSSION

From these 24 proteins, we predicted the WB value for about two thirds of the population. Under these conditions, the correlation coefficient between predicted and measured WB values is presented on Figure 1 (a and b).

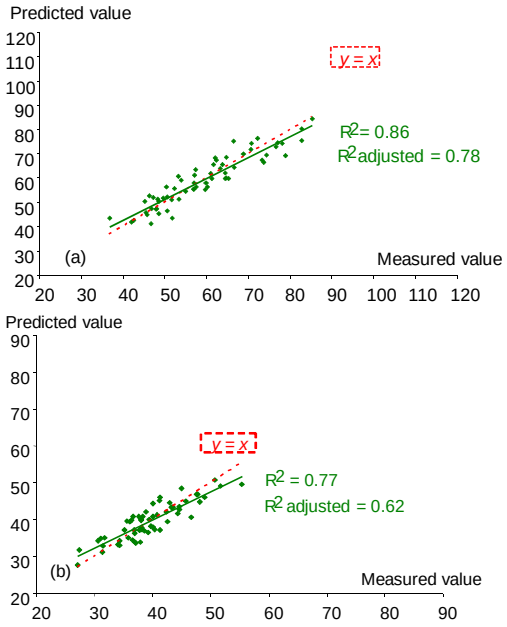

Figure 1 Equations of prediction of beef tenderness for the two muscles from the abundance of the 24 proteins analysed.

The proteins the most explanatory of tenderness and their respective coefficients in the equation are presented in Table 1.

In terms of tenderness markers on the ST muscle, the PRDX6 (cis-peroxiredox-6), LDHB (lactate dehydrogenase B), Hsp701B, Hsp70-GRP75 (Heat Schok Protein) and MyHC (Myosin Heavy Chain) II (IIa + IIx) were the variables most explicative of WB. In the LT muscle, PRDX6, Hsp20, Hsp70-GRP75 and αB-Crystallin (CRYAB) were the most explicative (Table 1).

Table 1 Equation parameters for the proteins significantly involved in tenderness prediction for the 2 muscles *Longissimus thoracis* (LT) and *Semitendinosus* (ST)

ST muscle			**LT muscle**		
Protein's name	Coefficient	P-value	Protein's name	Coefficient	P-value
PRDX6	2.09	<0.0001	CRYAB	-0.35	0.03
Hsp701A	1.62	0.001	PRDX6	1.12	0.001
LDH B	-1.76	<0.001	MyHC I	0.62	0.018
MyHC II	0.35	<0.01	Hsp20	0.97	<0.001
Hsp70-GRP75	1.54	0.03	Hsp70 GRP75	-0.74	0.04

These predictive equations demonstrated that PRDX6 protein is the only marker common to the two muscles, explaining the value of WB in the same way. This enzyme is involved in the fight against oxidative stress which is caused by free radicals of oxygen, resulting in formation of protein aggregates (Morzel et al 2006). These aggregates are disadvantageous to the development of tenderness.
Proteins of small Hsp family (Hsp27, Hsp20 and αB-Crystallin) are known to prevent the formation of these aggregates. Thus, the positive relationship between these Hsp and tenderness is quite concordant with the negative relationship between oxidative stress (PRDX6). This relationship with tenderness was retrieved and confirmed by Pearson

correlation analyses between tenderness (WB scores) and each protein abundance (data not shown).
Other HSPs are important in tenderness: these are HSP70, identified as negative markers, in contrast to HSP20. Indeed, these proteins also sequester pro-apoptotic factors such as BCL-2 inhibits apoptosis (Beere and Green 2001). These proteins also have chaperone function, but not on protein structure. This explains why in contrast to HSP20, the HSP70 are negative markers of tenderness in muscle ST.

This shows that oxidative stress plays an important role in predicting tenderness. The oxidative cellular pathways could be important and pertinent ways implied in tenderization processes. Futures studies for tenderness prediction models must integrate this aspect.
As the Heat Shock Proteins are also involved in apoptosis control, this cellular death mechanism appears to be important for tenderness. Moreover, apoptosis is closely linked with oxidative stress. In this study, we bring elements witch confirmed the theory of Ouali et al (2006), whose hypothesized that apoptosis is a early event in tenderization process.
The contractile protein, MyHC isoforms and the glycolytic enzyme LDHB, are heavily involved in the equation of ST muscle. But the lack of involvement of proteins of the same pathway did not allowed for interpretations of their precise roles in tenderization. Further studies on proteins of muscular or glycolysis pathways and their relationship with tenderness could bring answers.

IV. CONCLUSIONS

This work shows that from the abundance of 24 proteins we are able to predict the tenderness of about 60% of samples analyzed for each of the ST and LT muscles. We therefore confirm the important role of certain proteins in the tenderness and their relevance in the futures to a practical test. The results show that a relevant prediction model of this complex trait, meat tenderness, is becoming a reality and open new prospects for further studies.

This study also demonstrates that oxidative stress and apoptosis are relevant cellular mechanisms for tenderization processes. Their study could be an important perspective for further tenderness studies and genetic tests improvements.

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REFERENCES

1. Beere H. M. and Green D. R. (2001) Stress management - heat shock protein-70 and the regulation of apoptosis. Trends in Cell Biology 11 (1): 6-10.
2. Bernard C., Cassar-Malek I., LeCunff M., Dubroeucq H., Renand G., Hocquette J. F. (2007) New indicators of beef sensory quality revealed by expression of specific genes. J. Agric. Food Chem. 55: 5229-5237.
3. Guillemin N., Cassar-Malek I., Hocquette J. F., Jurie C., Micol D., Listrat A., Levéziel H., Renand G., Picard B. (2009a). La maîtrise de la tendreté de la viande bovine : Identification de marqueurs biologiques.INRA Prod. Anim. 22 : 331-344.
4. Guillemin N., Meunier B., Jurie C., Cassar-Malek I., Hocquette J. F., Levéziel H., Picard B. (2009b). Validation of a Dot-Blot quantitative technique for large-scale analysis of beef tenderness biomarkers. J. Physiol. Pharmacol. 60, 91-97.
5. Hocquette J. F., Laville E., Bernard C., Picard B., Cassar-Malek I., Sayd T., Lepetit, J., Jurie C., Meunier B., Micol D., Levéziel H., Renand G. (2008). Mise en évidence de marqueurs de tendreté de la viande bovine par des approches de génomique fonctionnelle. 12èmes Journées des Sciences du Muscle et Technologies de la viande, Tours
6. Morzel M., Gatellier P., Sayd T., Renerre M. and Laville E. (2006). Chemical oxidation decreases proteolytic susceptibility of skeletal muscle myofibrillar proteins. Meat Science 73 (3): 536-543.
7. Ouali A., Herrera-Mendez C. H., Coulis G., Becila S., Boudjellal A., Aubry L. and Sentandreu M. A. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. Meat Science 74 (1): 44-58.