Biomarkers of tenderness and intramuscular fat in five muscles from French PDO Maine-Anjou: I- Muscle type effect

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1 – MUSCLE TYPE EFFECT

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Abstract – This paper describes the difference in the relative abundance of 20 proteins in five muscles: *Longissimus thoracis* (LT), *Rectus abdominis* (RA), *Semimembranosus* (SM), *Semitendinosus* (ST) and *Triceps brachii* (TB) of cattle from the French Maine Anjou Protected Designation of Origin (PDO). These proteins are biomarkers of tenderness and intramuscular fat previously identified by proteomic analysis. The relative abundance of the proteins was evaluated by Reverse Phase Protein Array (RPPA) with specific antibodies. The main results showed a muscle effect on 16 of 20 biomarkers. The RA muscle was significantly different from other muscles by the lower abundance of 6 proteins and the higher abundance of 5 proteins. In the ST muscle, 4 proteins were less abundant and 4 others corresponding to fast glycolytic type, were more abundant. Clustering analysis revealed associations between proteins irrespective of muscle type and others were muscle dependent. The knowledge of these associations are important to understand the mechanism involved in these meat properties.

Key Words – proteins, proteomics, bovine, meat quality, adiposity.

I. INTRODUCTION

Beef tenderness is an essential sensory quality for the consumer who is willing to pay more to have a better meat tenderness. In addition, a lipid content of 3 to 4% in beef is required for a good sensory appreciation by consumers [1]. Currently, these criteria are evaluated only after slaughter by sensory analysis panel and/or mechanical measurements for tenderness, and by chemical analyzes for the lipid content. These methods are time consuming and costly. For several years, numerous research projects have identified proteins as potential biomarkers of tenderness [2] and more recently of meat adiposity. The aim of this study was to analyze the muscle effect on the abundance of these biomarkers identified in PDO Maine Anjou cattle.

II. MATERIALS AND METHODS

Five muscles: *Longissimus thoracis* (LT), *Semimembranosus* (SM), *Rectus abdominis* (RA), *Triceps brachii* (TB) and *Semitendinosus* (ST), from 101 PDO Maine Anjou cattle were sampled after slaughter in an industrial abattoir. Muscle samples were frozen in liquid nitrogen and stored at -80°C until analysis. The relative abundance of 20 proteins biomarkers of tenderness and/or intramuscular fat was measured on the 5 muscles by the Reverse Phase Protein Array (RPPA) which allows the simultaneous analysis of 500 samples for 20 proteins using specific antibodies [3]. The specificity of the 20 antibodies on bovine muscle and their conditions of use have been previously defined by western blotting which uses the same technical principle as the RPPA method. The muscle effect, was studied by ANOVA and was illustrated by principal component analysis (PCA). A hierarchical clustering of the 20 biomarkers was constructed using a combination of two dimension reduction approaches implemented in R software as described in Ellies-Oury *et al.* [4].

III. RESULTS AND DISCUSSION

Results of ANOVA and PCA clearly showed that four proteins: HSP40, FHL1 (Four and a half LIM domains protein 1), PYGB (glycogen phosphorylase B) and MDH1 (Malate dehydrogenase) did not differ between the 5 muscles. The muscles the most different from others were RA and ST (Fig. 1). The RA muscle was characterized by a significantly lower abundance of ENO3 (Enolase 3), PGK1 (Phosphoglucomutase 1), ALDOA (Aldolase), MyHC-IIX (Myosin heavy chain IIX), MLC1F (Fast myosin light chain 1), TPI1 (triosephosphate isomerase 1). On the contrary HSP27,

HSP70-1A1, CRYAB (alphaBcrystalin), TNNT1 (Troponin T slow), ALDH1A1 (Aldolase dehydrogenase 1) were higher abundant in the RA. The ST muscle was characterized by higher abundances of ENO3, PGK1, ALDOA, MyHC IIX and lower abundances of TTN (Titin), Hsp20, Hsp701A1, CRYAB. These data are in coherence with the knowledge about the contractile and metabolic properties of these muscles.

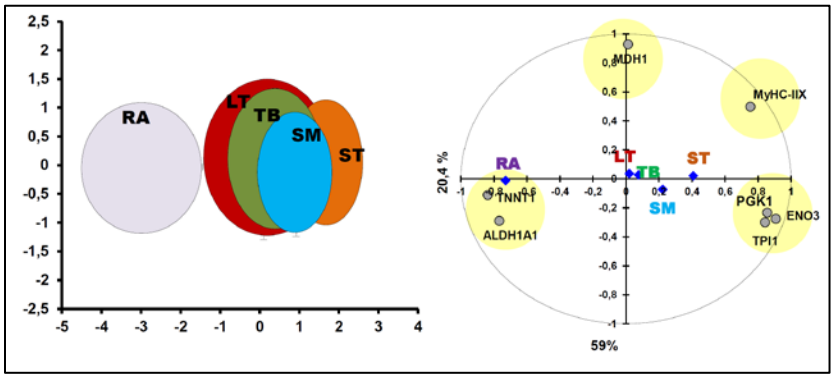

Figure 1. Principal component analysis of the 5 muscles (left) performed using the 7 surrounded proteins (right) representative of metabolic and contractile properties.

A clustering on variables (biomarkers) [4] (not shown) revealed that in the 5 muscles the 3 proteins TTN, FHL1 and MLC1F were present in the same cluster, FHL1 and MLC1F being positively associated together and negatively with TTN. ENO3, PGK1 and TPI1 were positively associated in another cluster in all muscles except in ST for which TPI1 was not associated to ENO3 and PKG1 was absent in the cluster. The three small HSP (20, 27, CRYAB) and TNNT1 were positively associated in a cluster in the most tender muscles (LT, SM, RA). However, in the two other muscles, less tender, TB and ST, CRYAB was negatively associated to ENO3 and PGK1 in a cluster. Moreover, MyHC-IIX was negatively associated with small HSP (20, 27, CRYAB) and TNNT1 in LT and RA. These two muscles are characterized by slow oxidative properties comparatively to TB, SM, ST (Fig.1). In SM and ST muscles, the most fast glycolytic (Fig.1), MyHCIIX was positively associated with MDH1 in a cluster.

IV. CONCLUSION

These data give new insights about the relationships between proteins biomarkers of tenderness and intra-muscular fat in several muscles differing by their contractile and metabolic properties, and characterized by different sensory qualities. These knowledge are important to understand the mechanisms involved in these meat properties.

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