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Abstract

This meta-proteomics review focused on proteins identified as candidate biomarkers of beef tenderness by comparing extreme groups of tenderness using two-dimensional electrophoresis (2-DE) associated with mass spectrometry (MS). We reviewed in this integromics study the results of 12 experiments that identified protein biomarkers from two muscles, *Longissimus thoracis* (LT) and *Semitendinosus* (ST), of different types of cattle: young bulls, steers or cows from beef breeds (Charolais, Limousin, Blond d'Aquitaine), hardy breed (Salers) or mixed breed (PDO Maine-Anjou). Comparative proteomics of groups differing in their tenderness evaluated by instrumental Warner-Bratzler shear force (WBSF) or by sensory analysis using trained panelists, revealed 61 proteins differentially abundant ($P < 0.05$) between tender and tough groups. A higher number of discriminative proteins was observed for LT (50 proteins) compared to ST muscle (28 proteins). The Gene Ontology annotations showed that the proteins of structure and contraction, protection against oxidative stress and apoptosis, energy metabolism, 70 family HSPs and proteasome subunits are more involved in LT tenderness than in ST. Amongst the list of candidate biomarkers of tenderness some proteins such as HSPB1 are common between the 2 muscles whatever the evaluation method of tenderness, but some relationships with tenderness for others (MYH1, TNNT3, HSPB6) are inversed. Muscle specificities were revealed in this meta-proteomic study. For example, Parvalbumin (PVALB) appeared as a robust biomarker in ST muscle whatever the evaluation method of tenderness. HSPA1B seems to be a robust candidate for LT tenderness (with WBSF) regardless the animal type. Some gender specificities were further identified including similarities between cows and steers (MSRA and HSPA9) in contrast to bulls. The comparison of the 12 proteomic studies revealed strong dissimilarities to identify generic biomarkers of beef tenderness. This integrative analysis allowed better understanding of the biological processes involved in tenderness in two muscles and their variations according to the main factors underlying this quality. It allowed also proposing for the first time a comprehensive list of candidate biomarkers to be evaluated deeply to validate their relationships with tenderness on a large number of cattle and breeds.

Keywords: Integromics; Mass spectrometry; Proteomics; Meat; Cattle; Biological mechanisms

1. Introduction

Beef tenderness is considered as the most important quality trait for consumer acceptability of fresh meat (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). This quality trait strongly influences consumer satisfaction and thus the repeat of purchase, particularly of beef, at the point of sale (Grunert, Bredahl, & Brunsø, 2004; Troy & Kerry, 2010). It is well documented that the origin of tenderness is multifactorial (Gagaoua, Monteils, & Picard, 2019; Ouali et al., 2013). It depends on the structural and biochemical properties of the muscles that develop and evolve throughout the life of the animal under the influence of factors related to the animal itself (age, sex, breed) and its rearing practices (Gagaoua, Picard, & Monteils, 2019; Gagaoua, Picard, Soulat, & Monteils, 2018; Listrat et al., 2016). Furthermore, processes such as *post-mortem* rigor development and ageing are dependent on these factors and could be modified by pre-slaughter (such as diet, handling, temperament, and stress), slaughtering and *post-mortem* conditions (pH and temperature decline, chilling regime, hanging method and carcass handling). This diversity of factors from the farm-to-fork (Gagaoua, Monteils, Couvreur, & Picard, 2019; Gagaoua, Picard, et al., 2018) explains why this quality trait is very variable and why there is difficulty to control or to predict it accurately despite the many research works carried out since decades.

Several technologies such as electrical stimulation, pelvis hung, tenderstretch, tendercut, pulsed electric field or super stretch (Bhat, Morton, Mason, & Bekhit, 2018; Y. H. B. Kim, Warner, & Rosenvold, 2014; Sørheim & Hildrum, 2002; Suwandy, Carne, van de Ven, Bekhit, & Hopkins, 2015; Warner et al., 2017) have been proposed to control the *post-mortem* conversion of muscle into meat and consequently increase tenderness. However, these technologies are not always used by industrials. Moreover, the available techniques to measure tenderness can only be applied after the death of the animal on aged meat, and they cannot be used online and in serial time on large number of samples. Today, tenderness is evaluated by sensory or mechanical analysis and the relationship between these two methods is variable (Gagaoua, Bonnet, Ellies-Oury, De Koning, & Picard, 2018). It is therefore necessary for both stakeholders and researchers to develop simple and fast tools applicable at slaughter or on the living animal, to evaluate tenderness. This type of tool could have applications to evaluate the potential tenderness of an animal or a cut of meat, to manage its marketing according to this potential and to develop genetic selection schemes based on this quality trait. For this purpose, numerous works, such as OMICs have been proposed during the last two decades.

Genomics has been used to discover and identify without *a priori* biomarkers of tenderness by applying approaches developed in medicine (Rifai, Gillette, & Carr, 2006). A biomarker can be defined as a measurable indicator of a specific biological state. Among OMICs tools, proteomics offers large interest to identify biomarkers because proteins and their enzymes entities determine the phenotypic diversity arising from a set of common genes. Furthermore, posttranslational modifications (PTMs) revealed by proteomics regulate structure, function, localization, maturation and turnover of proteins. Accordingly, Rifai and co-workers proposed a specific proteomic pipeline to identify biomarkers for clinical use, diagnosis or to monitor the activity of diseases and guide molecularly targeted therapy or assess therapeutic response (Rifai et al., 2006). This pipeline is composed of 6 steps « discovery, qualification, verification, research assay optimization, clinical validation and commercialization ». Therefore, the same pipeline was adapted by meat scientists for the discovery of beef tenderness biomarkers (Picard & Gagaoua, 2017). The first phase (**Figure 1**) is the « discovery » considered by Rifai et al. (2006) as "an unbiased semi-quantitative process by which the differential expression of specific proteins between states is first defined". The products of the discovery phase are lists of twenty to hundred proteins found to be differential between the two compared situations or conditions. These putative differentially abundant proteins can be considered as candidate biomarkers of the studied trait. Then, the relationships between the abundances of the putative biomarkers and the trait(s) of interest (phenome: a set of phenotypes) have to be evaluated with specific tools (Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Lopez-Pedrouso et al., 2019; Picard, Gagaoua, Al Jammas, & Bonnet, 2019; Picard et al., 2014). At the end, the validated biomarkers may be selected for the development of diagnostic test for commercialization (Kraus et al., 2011; Pierzchala et al., 2014; Marinus FW Te Pas, Hoekman, & Smits, 2011; M. F. Te Pas et al., 2013; Woelders, Te Pas, Bannink, Veerkamp, & Smits, 2011).

The aim of the present meta-proteomics was to review for the first time the studies conducted under identical laboratory conditions during the last decade to identify candidate protein biomarkers of beef tenderness by comparing groups of samples of high *versus* low tenderness for a given muscle from cattle raised in similar conditions. These studies have been applied mainly in *Longissimus thoracis* (LT) muscle but also in *Semitendinosus* (ST) from young bulls, steers and cows grouping different breeds. Groups of meat samples differing in their tenderness have been constituted on the basis of sensory analysis or mechanical measurements (Warner-Bratzler Shear force) according to each proteomic study.

Thus, two-dimensional electrophoresis (2-DE) combined with mass spectrometry to separate and identify the proteins of the tender *versus* tough meat samples were used. We report in this meta-proteomic a comprehensive list of specific or common candidate biomarkers to several muscles and genders. These data are firstly a source of new knowledge to better understand the biological processes and pathways involved in beef tenderness and also a list of candidate biomarkers of tenderness that once validated can be proposed to develop routine evaluation tools according to the pipeline of biomarkers discovery. Furthermore, these data are the first to highlight the major protein biomarkers of beef tenderness that can be identified whatever the treatments and techniques cited above.

2. Brief description of the experimental designs

This meta-proteomics work integrated 12 studies with a total of 120 samples that were selected based on their tenderness (tender *versus* tough) from 5 different experimental designs (**Table 1**) that are all fully described in previously published trials (Aviles et al., 2014; Chaze et al., 2013; Couvreur, Le Bec, Micol, & Picard, 2019; Gagaoua, Monteils, Couvreur, & Picard, 2017; Guillemin, Jurie, et al., 2011; Jurie et al., 2005; Morzel, Terlouw, Chambon, Micol, & Picard, 2008; Picard, Chambon, Meunier, Jacob, & Jurie, 2006).Those studies are briefly summarized in the supplementary data of **Table S1**. The samples grouped different animal types (young bulls (15 – 17 months), cows (67 or 86 months) and steers (30 months)) and two divergent muscles (*Longissimus thoracis* (LT) and *Semitendinosus* (ST)) differing in their contractile and metabolic properties (Ouali et al., 2005; Picard et al., 2014) sampled from the main French breeds (**Table 1**).

For each proteomic study, LT, a mixed fast oxido-glycolytic muscle sampled from 6th and 7th rib and ST, a mixed fast glycolytic muscle sampled from the center of the muscle, were excised from the carcasses 15 min p-m for 6 studies (1, 2, 5, 9, 10 and 12), 60 min p-m for 2 studies (3 and 11) and 24h p-m for 4 studies (4, 6, 7 and 8) as described in **Table 1**. The muscle samples were immediately frozen in liquid nitrogen and stored at -80°C until protein extractions for proteomic analysis.

For sensory analysis or mechanical measurements (WBSF) of tenderness, other muscle samples were taken at 24 h p-m, and aged for 14 (for 9 studies) or 21 days (for 3 studies) at 4°C (**Table 1**), then the chilled muscles were cut into steaks of about 1.5 cm thick, packed under vacuum and kept frozen at -20°C until analysis as previously described (Gagaoua, Bonnet, Ellies-Oury, et al., 2018).

3. Pipeline of beef tenderness biomarkers discovery

The standard proteomic workflow described in Picard and Gagaoua (2017) for the identification and discovery of protein biomarkers of beef tenderness was followed in this meta-proteomics (**Figure 1**). Briefly and according to the large literature, the main strategy that includes 6 steps used for the identification of biomarkers of meat quality including tenderness is based on comparative proteomics (Picard, Gagaoua, & Hollung, 2017). Therefore, two-dimensional electrophoresis (2-DE) (for review: (Görg, Weiss, & Dunn, 2004)) has been applied to analyze protein extracts from groups showing extremes (high *vs.* low) tenderness in order to reveal protein spots of interest with differential abundances. Statistical analyses and mass spectrometry, e.g., MALDI-TOF in this meta-proteomics (Jurinke, Oeth, & van den Boom, 2004) were then used to identify the corresponding proteins by the interrogation of specific bovine databases. When the candidate biomarkers have been identified, the next step is the evaluation and validation in large scale using several techniques such as immunobased techniques including Dot-Blot (Gagaoua, Terlouw, Boudjellal, & Picard, 2015; Gagaoua, Terlouw, Micol, et al., 2015; Guillemin, Bonnet, Jurie, & Picard, 2011; Guillemin et al., 2009), Reverse Phase Protein Arrays (Gagaoua, Bonnet, De Koning, & Picard, 2018; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Picard et al., 2019) or targeted proteomic methods such as selected reaction monitoring (SRM) or sequential window acquisition of all theoretical spectra (SWATH) (Wu, Dai, & Bendixen, 2019).

Step 1

The first step of the pipeline of beef tenderness biomarkers discovery is the selection of extremes (contrasting) sample groups (**Figure 1**). According to the experimental design and facilities of each study, we used sensory panelists to assess meat cooked at end-point cooking temperature of 55°C as usual in France (Gagaoua, Terlouw, Richardson, Hocquette, & Picard, 2019) using an unstructured scale from 0 to 10 or Warner-Bratzler shear force on cooked meat as a routine instrumental measure for tenderness to select extreme tender (TE) and tough (TO) samples from each experiment population. The details of the evaluation methods of tenderness are briefly summarized in the supplementary data of **Table S2**. The two techniques were applied on the whole 120 meat cut samples as described in previous studies (Dransfield et al., 2003; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Gagaoua, Micol, et al., 2016; Wheeler, Shackelford, & Koohmaraie, 1996) to select a minimum of 2 samples per tenderness group for studies 9, 10 and 12; 3 for studies 1 and 11; 4 for studies 2 and 5; 5 for studies 3 and

4; and 10 for studies 6, 7 and 8 (**Table 1**). The number of samples taken in each study varied from one project to another depending on the funding's and objectives of each research project. WBSF alone (mostly evaluated in N/cm² and in kg for study 3 only) was used to evaluate the meat samples of studies 1 – 5, sensory panelists alone were used for studies 9 – 12 and both sensory and instrumental methods were used for studies 6 – 8.

Step 2

The second step is sample and protein extracts preparation (**Figure 1**). Briefly, muscle proteins were extracted following the same procedure using frozen samples (Bouley, Chambon, & Picard, 2004). The frozen muscle tissue was homogenized in a lysis buffer containing 8.3 M urea, 2 M thiourea, 1% DTT, 2% CHAPS and 2% IPG buffer pH 3 – 10 and centrifuged at 10000 g for 30 min. The supernatant was harvested and the concentration of extracted protein was determined using the 2-DE Quant kit (Amersham Uppsala, Sweden). All protein samples were handled at 4°C to minimize the proteolytic activity.

Step 3

The protein extract samples were then subjected in the third step to 2-DE analysis following the same protocol (Bouley et al., 2005; Picard et al., 2016). Briefly, 800 µg of proteins were first subjected to isoelectrofocalisation (IEF) on 18 cm length IPG strips pH range 4 – 7 (Amersham-Biosciences, Uppsala, Sweden) in a Multiphor II gel apparatus at a temperature of 20.5°C. After a desalting step (50V, 7h), proteins were separated according to the following conditions: 200V for 1h, 200V increasing to 8000V over 5h, 8000V continuously until 73500Vh. Then, proteins were separated in the second dimension by SDS-PAGE on 12%T, 2.6%C separating polyacrylamide gels using Hoefer DALTsix system. 2-DE gels from the whole 12 studies were stained with G250 Colloidal Coomassie Blue for 72h. After staining, gels were rinsed for 1h in fresh distilled water before digitization. Triplicate gels from each protein extract were analyzed. Spot detection and quantification were performed with ImageMaster 2D Elite software on scanned gels (scanner UMAX, Amersham Pharmacia Biotech) at 300 dots per inch (dpi). Image warping, spot detection and volume quantification were realized using the SameSpots v3 software (Nonlinear Dynamics, Newcastle, UK). For each experiment, a gel corresponding to tender meat was selected as reference. 2-D gel data for each study were normalized by dividing each spot volume by the total volume of all the matched spots in the 2-D gel image, to obtain a normalized spot volume value. The proteins differentially abundant between tenderness groups were identified

by mass spectrometry using MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-time-of-flight).

Step 4

The fourth step of the pipeline is the statistical comparisons of the protein spots between groups. Accordingly and for each proteomic study, the electrophoretic profiles of the two groups of tenderness (TE and TO) were all compared using the statistical approach described in Meunier et al. (2005). It is based on the use of the Significance Analysis of Microarray (SAM) method based on a modified *t*-test. This approach considers not only the individual variability but also the average variability of proteins with the same level of expression than the considered protein. Among the differentially abundant proteins between the 2 groups of tenderness, only the proteins with a fold change >1.5 were retained (ratio of abundance levels between the 2 groups).

Step 5

In the fifth step, significantly differential spots were carefully excised by hand from at least three different replicate gels and placed in clean Safe-Lock Tube (Eppendorf, Hamburg Germany). Each tube allowed spot distaining and acetonitrile (ACN) drying. First, the spots were washed in 25 mM ammonium bicarbonate 5% ACN for 30 min and twice in 25 mM ammonium bicarbonate 50% ACN for 30 min each. The spots were then dehydrated with 100% ACN.

Step 6

In the last and sixth step, and for protein identification by MALDI-TOF, the dried gels were re-swelled in 25 mM ammonium bicarbonate and digested at 37 °C for 5 h with 10 to 15 µL (depending on the gel volume to be treated) of trypsin solution (12 ng/µL; V5111, Promega, Madison, WI, USA). Generally, 8 to 12 µL of ACN (depending on gel volume) was added to extract the peptides. Trypsin digested peptides solution was diluted in equal amount of matrix (5 mg/mL alpha-cyano-4-hydroxycinnamic acid (CHCA) in 50% ACN/0.1% Trifluoroacetic acid (TFA)). Peptide mass fingerprints (PMF) of trypsin-digested spots were determined in a positive reflectron mode using a Voyager DE Pro MALDI-TOF (Perspective Biosystem, Farminghan, MA, USA). External calibration was performed with a standard peptide solution (Proteomix, LaserBio Labs, Sophia-Antipolis, France). Internal calibration was performed using peptides resulting from autodigestion of porcine trypsin with protonated

masses of 842.509, 1045.564, and 2211.104. PMF were compared to *Bos taurus* Swiss Prot database or to other mammalian using MASCOT software. PMF of unidentified proteins (using bovine database) were compared to the nrMammalia database. The search criteria used were one missing trypsin cleavage site, partial methionine oxidation, partial carbamidomethylation of cysteine, and a mass deviation lower than 30 ppm. We required at least four matched peptides per protein for identification, using MASCOT probabilistic scores and accuracy of the experimental theoretical isoelectric point (p*I*) and molecular weight (MW). Protein spots with more than one protein identified in the mixture were considered for biological interpretation only if they passed both of the following criteria: i) the relative abundance based on the number of sequenced peptides per protein (EmPAI protein content (mol%) for the most abundant protein was larger than 50%; and, moreover; ii) the ratio of the EmPAI for this most abundant protein to EmPAI for the second most abundant protein in the spot mixture was at least 2.

4. Meta-proteomics database description and bioinformatics

Across the 12 proteomic experiments, a total of 61 putative protein biomarkers (unique gene names) of beef tenderness from young bulls, cows and steers grouping different continental breeds evaluated by sensory panelists or WBSF on *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles were differentially abundant ($P < 0.05$) between tender and tough groups (**Table 2**). Considering the 12 experiments, the number of differential proteins between extreme tenderness groups (**Table 2**) varies from 3 (study 12, ST muscle from young Salers bulls categorized by sensory panelists) to 22 proteins (study 2, LT muscle from young Charolais bulls categorized by WBSF). A higher number of putative protein biomarkers was observed for LT muscle (n = 50) compared to ST with 28 differential proteins (**Table S3** and **Table S4**). Panther screening of the whole proteins allowed their clustering into 6 biological pathways (**Table 2**):

i) Catalytic, metabolism & ATP metabolic process (n = 16): CKM, ENO1, ENO3, GAPDH, IDH1, MDH1, LDHB, PDHB, **PGM1**, PYGM, **TPI1**, GPD1, AKR1B1, SUCLA2, COX4I1 and **NDUFS1**.

ii) Contractile & associated proteins (n = 15): ACTA1, CAPZA3, CAPZB, MYH7, **MYH1**, **MYL2**, **MYBPH**, **MYL1**, MYRL2, MYL3, **TnnT3**, **TnnT1**, DES, **WDR1** and COL4A1.

iii) Chaperones & heat shock proteins (n = 11): HSPA9, HSPA5, **HSPB6**, **HSPB1**, HSPA2, HSPA8, HSPA1A, HSPA1B, **STIP1**, **CRYAB** and **YWHAE**.

iv) Binding, cofactor & transport proteins (n = 11): ACBD5, ANKRD2, APOBEC2, DBI, GDI2, **ALB**, RABGGTA, TRIM72, AHCY, SRL and PVALB.

v) Oxidative stress & cell redox homeostasis (n = 6): GSTP1, SOD1, PRDX6, PARK7, MSRA and **PDIA3**.

vi) Proteasome & associated proteins (n = 2): PSMB2 and PSMC2.

Among this list of 61 biomarkers, 17 proteins given in bold character above (PGM1, TPI1, NDUFS1, MYH1, MYL2, MYBPH, MYL1, TnnT3, TnnT1, WDR1, HSPB6, HSPB1, STIP1, CRYAB, YWHAE, ALB and PDIA3) are common to the two studied muscles.

Gene ontology annotations of proteomic data was realized using the ProteINSIDE workflow tool (Kaspric, Picard, Reichstadt, Tournayre, & Bonnet, 2015) to analyze the 61 proteins and gather biological information provided by functional annotations. The protein-protein interactions (interactomics) of biological function of the proteins by muscle or for both LT and ST muscles were realized using STRING webservice database (http://string-db.org/). Default settings of confidence of 0.5 and 4 criteria for linkage: Co-occurrence, experimental evidences, existing databases and text mining were used. Furthermore, the ProteQTL tool included in ProteINSIDE (http://www.proteinside.org) was used to search for tenderness Quantitative trait loci (QTL) among the list of putative protein biomarkers of tenderness. This tool interrogates a public library of published QTL in animal science: CattleQTLdb (Hu, Park, Wu, & Reecy, 2013) that contains cattle QTL and association data curated from published scientific papers.

The degree of similarity between the protein biomarkers among the 12 proteomic studies to explain tenderness was estimated as the percentage of proteins shared based on the *F* coefficient (López-Pedrouso, Bernal, Franco, & Zapata, 2014): $F = 2n_{xy}/(n_x + n_y)$. Where n_{xy} is the number of tenderness biomarkers shared by study x and y; and n_x and n_y are the total numbers of biomarkers retained in studies x and y, respectively (Gagaoua, Terlouw, et al., 2019). Then, a pairwise of biomarker distance (*D*) expressed as a percentage based on the abundances of the biomarkers under the different factors was built as estimated by $D = 1 - F$.

5. Gene Ontology annotations results of the protein biomarkers of beef tenderness

The corresponding Gene Ontology annotations: cellular components, biological pathways and molecular functions are illustrated in **Figure 2A,B** for LT and ST muscles, respectively.

The distributions of the molecular functions are deeply described in **Figure 3**. Considering the two muscles together, the most represented functions are: catalytic, metabolism and ATP metabolic process (26%) and contractile and associated proteins (25%) followed by chaperones and heat shock proteins (18%) (**Table 2** and **Figure 3**). Oxidative stress and cell redox homeostasis represents 12% of the biological functions in LT and only 4% in ST muscle (**Figure 3**). Proteasome and associated proteins were found only in LT muscle (4%).

The interactomes constructed with the differential proteins for each muscle illustrated the main biological functions (**Figure 4A,B**). The contractile proteins MYL1, MYL2, MYH1, TnnT1, TnnT3, WDR1 and MYBPH are common to both muscles. On the contrary, structural proteins such as DES, CAPZB, CAPZA3 and ACTA1 were differential proteins in only LT muscle. For chaperones and Heat shock proteins pathways, 3 small HSP (HSPB1, HSPB6 and CRYAB) as well as STIP1 and YWHAE were differential in both 2 muscles. However, proteins of the HSP 70 family: HSPA1B, HSPA1A, HSPA2, HSPA8 and HSPA9 were specific to LT muscle. HSPA5 was found differential in only ST muscle. Concerning the catabolic and metabolic pathways, 3 proteins (TPI1, PGM1 and NDUFS1) were differential in both LT and ST muscles. A large number of glycolytic and oxidative proteins were found differential and specific to LT muscle (**Figure 4A**), and two proteins: COX4I1 and SUCLA2 were differential only in ST muscle. The interactome in LT muscle illustrates numerous interactions among the different biological pathways and putative biomarkers. The oxidative stress and cell redox homeostasis in ST muscle is represented by only PDIA3 protein which was differential also in LT muscle with 5 other proteins: MSRA, GSTP1, PRDX6, PARK7 and SOD1. Among the proteins of the binding, cofactor and transport proteins, only ALB was common to the two muscles with connections with all other biological pathways whatever the muscle. The other proteins of this cluster are specific to the considered muscle (**Figure 4A,B**).

6. Description of the results for each factor of the meta-proteomics

As stated above, the number of proteins is variable from one study to another and was also found to be variable from one biological pathway to another (**Table 2** and **Figure 3**). The comparison of the common proteins among the 12 proteomic studies in terms of their redundancy revealed a strong dissimilarity based on the pairwise biomarker distance (**Figure 5**). The dissimilarity was particularly strong for muscles as there was an unbalance between the number of proteins identified for LT and ST muscle. The pairwise distance given in

Figure 5 highlights the strong dissimilarities to identify generic biomarkers of beef tenderness if we refer to the factors included in this study.

The direction of the putative protein biomarkers with tenderness is also variable (**Table 2, Table S3** and **Table S4**). Taken the 16 proteins belonging to catalytic, metabolism and ATP metabolic process together, 18 times positive and 14 times negative directions were observed between the proteins and tenderness for both LT and ST muscles and whatever the other factors (**Table 2**). For LT (13+, 11-) and ST (5+, 3-) muscles separately, the same trend was observed. From the second pathway of 15 contractile and associated proteins, a high number of associations was found for both muscles taken together with 23 times positive, 16 times negative and 2 times with both positive and negative directions. For LT muscle alone, there were 12 times positive, 13 times negative and 2 times both directions. For ST muscle, more positive (11 times) than negative (3 times) directions were found. In the third biological pathway of chaperones and heat shock proteins grouping 11 proteins, an equivalent 15 times associations of positive and negative directions was found with 5 times both signs depending of the isoform mostly of HSPB1 (4 times) and HSPB6 (1 time). For LT muscle alone, more negative association (12 times) than positive (9 times) were found with the same 5 in both directions. For ST muscle, few associations were found, with 6 times positive and 3 times negative associations with tenderness. In the fourth biological pathway grouping 11 binding, cofactor and transport proteins for the two muscles, 10 times positive and 5 times negative associations with tenderness were found (**Table 2**). For LT muscle, less association compared to ST were reported: 2 positive *vs.* 8 and 4 negative *vs.* 1, respectively. In the fifth pathway grouping 6 proteins belonging to oxidative stress and cell redox homeostasis process, 6 times positive and 5 times negative associations were reported for both muscles. Most of the associations were for LT muscle 5 times positive and 5 negative, compared to only 1 positive association for ST. finally, the 2 proteasome and associated proteins reported in this meta-proteomic were specific to LT muscle and were both positive.

6.1. Gender effect

In Charolais LT muscle and among 3 proteomic studies, 28 proteins were differentially abundant between groups of tenderness evaluated by WBSF: 22 proteins in young bulls, 9 in steers and 10 in cows (**Figure 6**). Only two proteins HSPA1B and TPI1 were found to be differentially abundant for the 3 genders. The abundance of HSPA1B was always lower in tender meat for the three genders. The abundance of TPI1 was lower in tender meat of young

bulls and higher in tender meat of steers and cows. These differences could be explained mainly by differences in sex hormones between the genders. Indeed, the effect of estrogens on skeletal muscle properties has been largely studied (Enns & Tiidus, 2010; Sauerwein & Meyer, 1989) with important regulation of energy metabolism pathways, including glucose transport, glycolysis and tricarboxylic acid cycle (Y. Xu & López, 2018).

On another hand, two proteins: TnnT3 and YWHAE were found differentially abundant only in males but inversely in steers and young bulls. In young bulls, TnnT3 was lower abundant in tender meat, while in steers it was higher. Two proteins MSRA and HSPA9 (GRP75) were differentially abundant in groups of divergent tenderness in steers and cows but not in young bulls. MSRA was higher abundant and GRP75 was lower abundant in groups of high tenderness. Five proteins were common to cows and young bulls: HSPB1 and MYBPH in the same direction, and ENO1, MYH1 and PGM1 in opposite direction. In cows, PGM1 and ENO1 were higher abundant in tender group and MYH1 (MyHC-IIx) was lower abundant, the opposite was observed in young bulls. ENO3 was higher abundant in tender LT only of cows.

6.2. Breed effect

Considering LT tenderness groups only evaluated by sensory panelists in young bulls from 3 breeds, a total of 26 proteins were found differentially abundant: 8 in Charolais (CH), 14 in Limousin (LI) and 15 in Blond d'Aquitaine (BA) (**Figure 7**). Two biomarkers, ACTA1 and HSPB1 were common to the three breeds. PGM1 and TnnT3 were lower abundant in tender LT in BA and CH but not different in LI. The oxidative protein PARK7 was higher abundant in tender LT in CH but lower abundant in LI and not different in BA breed. Four proteins were common to Li and BA: HSPB6, CRYAB, CAPZB and ENO3. This later protein was always lower in abundance for both LI and BA tender group and, the three other proteins showed inverse relations with tenderness.

In ST muscle, 17 proteins were differentially abundant between the groups of tenderness evaluated by sensory analysis in young bulls and 9 proteins were identified for Charolais and Blond d'Aquitaine, 4 for Limousin and 3 for Salers (**Figure 8**). This number of divergent proteins is lower than observed in LT muscle. Among the putative biomarkers of ST muscle, PVALB was found for CH, BA and LI breeds and never for Salers. Three proteins were common between CH and BA young bulls: HSPB1, MYL1 and TnnT3 were higher abundant in tender ST from these 2 breeds. This is coherent with earlier studies indicating that ST

muscle is tenderer when it is likely fast glycolytic oriented (Picard et al., 2014). HSPB1 was differently abundant between groups of tenderness but inversely between the two breeds. MYL2 was lower abundant and MYBPH higher abundant in tender ST in LI and CH breeds.

If we consider only the population of cows, from CH and PDO MA breeds, 21 putative protein biomarkers were found differently abundant between groups of tenderness evaluated by WBSF, 17 in PDO MA and 10 in CH cows (**Figure 9**). Six proteins, ENO1, ENO3, MYH1, HSPB1, HSPA1B and MSRA, were common to the two cow breeds. ENO1, ENO3 and MSRA were higher abundant and MYH1 and HSPA1B were lower abundant in the tender LT. HSPB1 was inversely related with tenderness in the two cow breeds. Trim72 was found differentially abundant between groups of tenderness in only PDO MA breed in which it was lower abundant in the tender LT steaks (**Figure 9**).

6.3. Muscle type effect

The comparisons of ST and LT muscles from young Charolais bulls allowed identifying a total of 34 differentially abundant proteins between groups of tenderness evaluated by WBSF (**Figure 10**). A total of 22 proteins were identified in LT *versus* 16 in ST. Among them, 4 biomarkers, TPI1, HSPB6, HSPB1 and YWHAE, were common to the two muscles. HSPB6 was higher abundant in the tender group whatever the muscle. The 3 others were inversely related with tenderness in the two muscles. TPI1 and YWHAE were higher abundant in tender ST and lower abundant in tender LT. The inverse was observed for HSPB1. An inverse relationship between some proteins such as HSPB1 and tenderness was already reported in the large literature. For example, the results illustrated in **Figure 10** highlighted that in LT muscle the proteins related with tenderness correspond mainly to contractile, structural and Heat shock proteins whereas in ST the proteins related with tenderness are mainly involved in metabolism in coherence with the Gene Ontology of **Figure 2**.

6.4. Effect of evaluation method of tenderness

For LT muscles of young Charolais bulls, 22 proteins were differentially abundant between groups of tenderness evaluated by WBSF and 8 between groups of tenderness evaluated by sensory panelists (**Figure 11**). Among a total of 25 proteins, 5 biomarkers, PGM1, ACTA1, TnnT3, HSPB1 and PARK7, were common to the two evaluation methods of tenderness. PGM1 and TnnT3 were lower abundant in tender LT whatever the methods used.

For ST muscles of young Charolais bulls, 16 proteins were differentially abundant between groups of tenderness evaluated by WBSF and 9 between groups of tenderness evaluated by sensory panelists (**Figure 12**). Three biomarkers, MYL2, HSPB1 and PVALB, were common to the two evaluation methods of tenderness. Among them, HSPB1 and MYL2 were lower abundant and PVALB higher abundant in the tender groups whatever the evaluation method.

7. Overall discussion

This integrative meta-proteomics study which gathered 61 proteins identified following the same procedure from one laboratory, provides new insights and robust results on the putative protein biomarkers of beef tenderness. It allowed us to identify the main proteins that are common to several muscles, animal types (gender) and evaluation method of tenderness, as well as those that are specific to each of these factors. This integrative review allows a better understanding of the biological pathways involved in beef tenderness that would explain its variability according to muscle, breed, gender or evaluation method of tenderness. For the two muscles considered in this review that are *Longissimus thoracis* (LT) and *Semitendinosus* (ST), a higher number of differential proteins discriminating tender *versus* tough meat groups were found for WBSF compared to sensory panelists. In the following discussion sections, we first compared the list of the candidate biomarkers (by biological family) identified in the 12 proteomic studies to those of the literature by linking each putative biomarker to some earlier proteomic studies and then, we focused on the major biomarkers candidates of great interest (reported more than 4 times in the 12 studies) deserving further evaluation and validation studies following the pipeline of tenderness biomarkers discovery (**Figure 1**).

7.1. Brief comparison of the list of the putative biomarkers to bovine proteomic studies from the large literature

During the last two decades, meat scientists used OMICs tools more precisely proteomics, to identify accurate biological markers of beef tenderness that would make possible the categorization of meat cuts soon after slaughter on the basis of their potential final tenderness and/or to propose those biomarkers to optimize the genetic selection of beef animals on the basis of this quality. As highlighted above and following the guidelines of several studies in the large literature (D'Alessandro, Rinalducci, et al., 2012; D'Alessandro & Zolla, 2013; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Gagaoua, Terlouw, et al., 2019; Longo, Lana, Bottero, & Zolla, 2015; Mullen, Stapleton, Corcoran, Hamill, & White, 2006; Ouali et al., 2013; Picard & Gagaoua, 2017; Picard et al., 2017; Picard et al., 2015; Picard, Lefevre, &

Lebret, 2012), 6 distinct but interconnected biological pathways were identified (Gagaoua, Terlouw, Boudjellal, et al., 2015; Guillemin, Bonnet, et al., 2011; Picard et al., 2018).

The 61 biomarkers identified in this meta-proteomics and their directions (positive, negative or both) with mechanical (WBSF) or sensory beef tenderness are given in **Table 2**. The discussion of the most interesting candidates according to the corresponding biological functions in which they are involved allow producing new insights about tenderness determinism. For instance, in the following sections, a brief description for each pathway and a comparison to the large literature are given. The directions will not be discussed in the following sub-sections and only non-exhaustive cattle references from the large literature for reported putative protein biomarkers by biological family are given.

7.1.1. Biomarkers belonging to catalytic, metabolism & ATP metabolic pathway

Among the list of the 16 putative protein biomarkers of this biological pathway, 14 were reported in earlier studies and two that are AKR1B1 (Aldo-keto reductase family 1 member B) and COX4I1 (Cytochrome c oxidase subunit 4 isoform 1, mitochondrial) were for the first time identified in this meta-proteomic. Of those reported in the large literature: CKM (Beldarrain et al., 2018; Bjarnadottir et al., 2012; Laville et al., 2009; Polati et al., 2012; Sierra et al., 2012; Zapata, Zerby, & Wick, 2009), ENO1 (D'Alessandro, Rinalducci, et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Polati et al., 2012), ENO3 (Bjarnadottir et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Marino et al., 2014; Polati et al., 2012; Zhao et al., 2014), GAPDH (D'Alessandro, Marrocco, et al., 2012; Laville et al., 2009; Mahmood, Turchinsky, Paradis, Dixon, & Bruce, 2018; Marino et al., 2014; Polati et al., 2012), IDH1 (Zapata et al., 2009), MDH1 (Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Jia et al., 2009), LDHB (Baldassini et al., 2015; Laville et al., 2009; Picard et al., 2014; Polati et al., 2012), PDHB (Grabez et al., 2015), PGM1 (Anderson, Lonergan, & Huff-Lonergan, 2014; Bjarnadottir, Hollung, Faergestad, & Veiseth-Kent, 2010; D'Alessandro, Rinalducci, et al., 2012; Laville et al., 2009), PYGM (Laville et al., 2009), TPI1 (D'Alessandro, Rinalducci, et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Grabez et al., 2015; Rosa et al., 2018), GPD1 (Bjarnadottir et al., 2010; Jia et al., 2007), SUCLA2 (Jia et al., 2006) and NDUFS1 (Chaze et al., 2013).

7.1.2. Biomarkers belonging to contractile & associated proteins

Among the list of the 15 putative protein biomarkers of this biological pathway, 13 were reported in earlier studies and two that are CAPZA3 (F-actin-capping protein subunit alpha),

and COL4A1 (Collagen alpha-1 (iv) chain) were for the first time identified in this meta-proteomic. Of those reported in the large literature: ACTA1 (Beldarrain et al., 2018; Bjarnadottir et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Laville et al., 2009; Polati et al., 2012; Zapata et al., 2009), CAPZB (Bjarnadottir et al., 2010; Picard et al., 2014), MYH7 (Grabez et al., 2015; Zapata et al., 2009; Zhao et al., 2014), MYH1 (Beldarrain et al., 2018; Picard et al., 2014; Polati et al., 2012; Zhao et al., 2014), MYL2 (Polati et al., 2012; Zapata et al., 2009), MYBPH (Guillemin, Bonnet, et al., 2011; Morzel et al., 2008; Picard et al., 2014), MYL1 (M. E. Carvalho et al., 2014; D'Alessandro, Marrocco, et al., 2012; D'Alessandro, Rinalducci, et al., 2012; Guillemin, Bonnet, et al., 2011; Rosa et al., 2018), MYRL2 (Bjarnadottir et al., 2012), MYL3 (Malheiros et al., 2019), TnnT3 (Beldarrain et al., 2018; Malheiros et al., 2019; Muroya et al., 2004; Rosa et al., 2018; Sierra et al., 2012), TnnT1 (D'Alessandro, Rinalducci, et al., 2012; Malheiros et al., 2019; Rosa et al., 2018), DES (Guillemin, Bonnet, et al., 2011; Malheiros et al., 2019; Zapata et al., 2009) and WDR1 (Chaze et al., 2013).

7.1.3. Biomarkers belonging to chaperones & heat shock proteins

Among the list of the 11 putative protein biomarkers of this biological pathway, 10 were reported in earlier studies and one only which is HSPA2 (Heat shock-related 70 kDa protein 2), was for the first time identified in this meta-proteomic. Of those reported in the large literature: HSPA9 (Grabez et al., 2015; Jia et al., 2009; Rodrigues et al., 2017), HSPA5 (Rodrigues et al., 2017), HSPB6 (D'Alessandro, Marrocco, et al., 2012; D'Alessandro, Rinalducci, et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Gagaoua, Terlouw, et al., 2019; Guillemin, Bonnet, et al., 2011; Polati et al., 2012; Zapata et al., 2009), HSPB1 (M. E. Carvalho et al., 2014; D'Alessandro, Rinalducci, et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Guillemin, Bonnet, et al., 2011; N. K. Kim et al., 2008; Polati et al., 2012), HSPA8 (D'Alessandro, Rinalducci, et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018), HSPA1A (Bjarnadottir et al., 2012; M. E. Carvalho et al., 2014; D'Alessandro, Rinalducci, et al., 2012; Gagaoua, Terlouw, et al., 2019), HSPA1B (Baldassini et al., 2015; D'Alessandro, Rinalducci, et al., 2012), STIP1 (Chaze et al., 2013), CRYAB (D'Alessandro, Rinalducci, et al., 2012; Guillemin, Bonnet, et al., 2011; Morzel et al., 2008; Polati et al., 2012; Zapata et al., 2009) and YWHAE (Chaze et al., 2013; D'Alessandro, Rinalducci, et al., 2012; Mahmood et al., 2018; Rodrigues et al., 2017).

7.1.4. Biomarkers belonging to binding, cofactor & transport proteins

Among the list of the 11 putative protein biomarkers of this biological pathway, only 4 biomarkers were reported in earlier studies and 7 which are ACBD5 (Acyl-CoA-binding domain-containing protein 5), ANKRD2 (Ankyrin repeat domain 2), APOBEC2 (Probable C->U-editing enzyme APOBEC-2), DBI (Acyl-CoA-binding protein), AHCY (Adenosylhomocysteinase), SRL (Sarcalumenin) and PVALB (Parvalbumin α) were for the first time identified in this meta-proteomics. Of those reported in the large literature: GDI2 (Chaze et al., 2013; Mahmood et al., 2018), ALB (Baldassini et al., 2015; Laville et al., 2009; Polati et al., 2012), RABGGTA (Chaze et al., 2013) and TRIM72 (Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Rosa et al., 2018).

7.1.5. Biomarkers belonging to oxidative stress & cell redox homeostasis

Among the list of the 6 putative protein biomarkers of this biological pathway, 4 were reported in earlier studies and two only which are MSRA (Mitochondrial peptide methionine sulfoxide reductas) and PDIA3 (Protein disulfide-isomerase A3), were for the first time identified in this meta-proteomic. Of those reported in the large literature: GSTP1 (Chaze et al., 2013), SOD1 (D'Alessandro, Marrocco, et al., 2012; Grabez et al., 2015; Marino et al., 2014), PRDX6 (Guillemin, Bonnet, et al., 2011; Jia et al., 2009; Zhao et al., 2014) and PARK7 (Jia et al., 2007; Jia et al., 2009; Mahmood et al., 2018; Picard et al., 2014; Polati et al., 2012).

7.1.6. Biomarkers belonging to proteasome & associated proteins

The 2 putative protein biomarkers of this biological pathway (PSMB2 and PSMC2), were both earlier reported to be related to beef tenderness (Chaze et al., 2013; Ouali et al., 2013).

7.2. Quest for quantitative trait loci (QTL) from the list of the putative protein biomarkers

Among the list of the 61 proteins candidate biomarkers of tenderness, the ProteQTL tool (http://www.proteinside.org/) revealed 15 of them (shown by blue ovals for both muscles in the networks) for which a QTL for WBSF or sensory tenderness has been reported (**Figure 4**). The 15 QTL proteins belong to the following biological functions:

i) Catalytic, metabolism & ATP metabolic process (n = 3): PYGM, LDHB and **PGM1**.

ii) Contractile & associated proteins (n = 4): **MYL2**, MYL3, CAPZB and CAPZA3.

iii) Chaperones & heat shock proteins (n = 4): HSPA2, HSPA9, HSPA8 and **STIP1**.

iv) Binding, cofactor & transport proteins (n = 3): PVALB, GDI2 and AHCY.

v) Oxidative stress & cell redox homeostasis (n = 1): GSTP1.

vi) Proteasome & associated proteins (n = 0): none.

The 3 putative biomarkers (PGM1, MYL2 and STIP1) given in bold character in this list are common to LT and ST muscles. The data of this meta-proteomics validated the important role that these 15 proteins would play in meat tenderness of cattle. This list of proteins needs further investigation in the evaluation and validation steps of the pipeline of beef tenderness biomarkers discovery (**Figure 1**), namely PGM1, MYL2, CAPZB, HSPA9 and PVALB that were identified in more than 3 proteomic studies and reported in earlier studies (Allais et al., 2011; Hao et al., 2016; Hu et al., 2013; Hwang et al., 2015; Ramayo-Caldas, Renand, Ballester, Saintilan, & Rocha, 2016; Sorbolini et al., 2015).

7.3. Major beef tenderness biomarkers common to LT and ST muscles

7.3.1. Important role of HSPB1 and HSPB6 in beef tenderness

Earlier meat proteomic studies whatever the quality trait and the species, have confirmed the implication of heat shock proteins (HSPs) including small, large and co-chaperones (For review: (Lomiwes, Farouk, Wiklund, & Young, 2014; Picard & Gagaoua, 2017; Picard et al., 2017)). HSPs are ubiquitous and evolutionarily conserved proteins that are mainly classified into 5 subfamilies on the basis of their molecular mass, *e.g.,* HSP60, HSP70, HSP90, and HSP100 and the small HSPs (MS: 12–43 kDa). These abundant and dynamically expressed proteins were for the first time suggested by Ouali et al. (2006) to play a role in muscle to meat transformation by decelerating *post-mortem* muscle apoptosis onset, and hence affecting the final quality of meat products. Although the exact underlying mechanisms in *post-mortem* muscle and in cooked meat are not yet understood, these superfamily of proteins appear to influence final tenderness through different mechanisms (Ouali et al., 2013; Picard & Gagaoua, 2017): **i)** modulation of initiators or effectors caspases activities (by inhibition); **ii)** protection of cellular structures against apoptosis (anti-apoptotic role); **iii)** myofibrillar protein protection (binding of protease cleavable substrate) from degradation by inhibition of proteolytic activity; **iv)** refolding of denatured proteins caused by pH decline and resultant protease activity dramatically altering protein integrity and native conformation; and last but not least **v)** maintenance of the correct conformation of proteins and preservation of their biological functions, folding and unfolding, and refolding of damaged protein entities.

From the most interesting HSPs proteins identified in this meta-proteomics and whatever the muscle, the small HSPB1 (HSP27) and HSPB6 (HSP20) proteins were reported 9 and 5 times respectively from the 12 studies. The former was identified in different breeds and irrespective of the evaluation method of tenderness and the later was specific to young bulls but found irrespective of the same factors of HSPB1 (**Table 2**). However, the relations with tenderness for the two sHSP were not always in the same direction, a phenomenon that we already reported in previous studies (Gagaoua, Terlouw, et al., 2019; Gagaoua, Terlouw, Monteils, Couvreur, & Picard, 2017; Picard & Gagaoua, 2017; Picard et al., 2014; Rosa et al., 2017).

Small HSPs are intracellular stabilizers that are dynamically expressed in muscle after slaughter to respond in specific regions of muscle cells to the irreversible denaturation and aggregation during heat stimulation of muscle proteins and thus maintain cellular homeostasis (for review: (Lomiwes et al., 2014)). For HSPB1, an inverse relationship with tenderness was observed by different authors between ST and LT or between breeds (Picard et al., 2014). On another hand, an earlier study by Chaze et al. (2009) using western-blotting on 2D-E gels revealed 12 different isoforms of HSPB1 and showed that the differential spots among groups of tenderness differed according to the breed considered, in coherence with the results of the present review. For instance, it seems that the relation with tenderness depends on the contractile and metabolic properties of the considered muscle (Picard et al., 2018) as their expression differ between oxidative and glycolytic muscle (Golenhofen, Perng, Quinlan, & Drenckhahn, 2004).

To better understand the role of this sHSP in meat tenderization, we produced null-*HSPB1* mice and investigated the effect on both the structure and the proteome of different muscles in comparison to their wild-types (Kammoun et al., 2016; Picard et al., 2016). The findings of these studies revealed that major modification of expressions of proteins are induced in *Gastocnemius* muscle (fast glycolytic) which most of them proposed in the present review as candidate biomarkers of tenderness: HSPA9, HSPA8, PARK7, TNNT3, TPI1, MDH1, PDHB, CKM, PYGM and ALB. From these original findings, one can speculate an interaction between all these proteins during meat tenderization. Moreover, *in silico* analysis identified the main interactors of HSPB1, among them YWHAE, also found differential between groups of tenderness (Guillemin, Bonnet, et al., 2011) and discussed below (sub-section 7.3.4). It is worthwhile to note, that differences in proteins interacting with HSPB1 were observed between *Gastrocnemius* (fast glycolytic) and *Soleus* (slow oxidative) muscles,

suggesting muscle-specific effects and thus the role that this protein would play in tenderness might differ from muscle cuts with different metabolic and contractile properties (Picard et al., 2014). Overall, these data suggest that HSPB1 seems to play a central role in beef tenderness determinism by interacting with five main protein families (HSPs, pro/anti-apoptotic factors, translation factors, cytoskeletal proteins, and oxidative proteins) in accordance with the interactomes and correlation networks of tenderness proposed by (Guillemin, Bonnet, et al., 2011) and (Gagaoua, Terlouw, Boudjellal, et al., 2015).

HSPB6, or HSP20, is another sHSP retained 5 times whatever the muscle for young bulls. Accordingly, it can be proposed as for HSPB1, as a potential biomarker of tenderness due to the role it can play in muscle to meat conversion (Christensen et al., 2017; Contreras-Castillo, Lomiwes, Wu, Frost, & Farouk, 2015; Lomiwes, Farouk, Frost, Dobbie, & Young, 2013; Lomiwes et al., 2014; Pulford, Frost, Lomiwes, & Farouk, 2008). As mentioned in sub-section 7.1.3, several studies identified HSP20 as a good biomarker of beef tenderness in proteomic studies, and other meat qualities such as color and pH decline (Gagaoua, Bonnet, De Koning, et al., 2018; Gagaoua, Couvreur, Le Bec, Aminot, & Picard, 2017). For example, in LT muscle of Angus steers, it was found divergently abundant between sample groups contrasting in their tenderness (Malheiros et al., 2019; Zapata et al., 2009). HSPB6 acts by protecting structural proteins, including desmin, actin and titin (Ghosh, Houck, & Clark, 2007).

Furthermore, in skeletal muscle, this protein protects against atrophy, ischemia, hypertensive stress, and metabolic dysfunction (Dreiza et al., 2010). As HSPB6 has a binding domain to troponin 1 (Rembold, Foster, Strauss, Wingard, & Eyk, 2000), it could affect skeletal muscle contraction through the troponin complex. Moreover, in obese subjects with decreased insulin sensitivity, it promotes blood flow and increases glucose utilization during exercise (Y. Wang, Xu, & Cooper, 1999). So, HSPB6 is involved both in muscle contraction and metabolism. Phosphorylation of HSPB6 also leads to the disruption of the actin cytoskeleton. Therefore, its role in tenderness could be through these biological functions in different types of muscles.

7.3.2. Troponin T Fast, a biomarker of tenderness irrespective of muscle and gender

TnnT3 was found differential between groups of tenderness in 7 of the 12 proteomic studies (**Table 2**). After HSPB1, it is the most frequently identified differential biomarker in this meta-proteomic review. In LT muscle of young bulls, TnnT3 was less abundant in tender

meat and the inverse was observed for steers. Similar to LT muscle of steers, a higher abundance in tender ST meat of young BA and CH bulls evaluated by sensory panelists was observed. A difference between genders was further observed for sensory tenderness as TnnT3 was less abundant in tender LT of young bulls but higher in steaks of PDO Maine-Anjou cows evaluated by WBSF as in CH steers. The *TNNT* gene encodes a protein that is a subunit of troponin, which embodies a regulatory complex on the thin filament of the sarcomere. Troponin T fast fragment was reported to play a role in the prevention of the formation of actomyosin ATPase complex (Fink, Momke, Wohlke, & Distl, 2008; Lehman, Galińska-Rakoczy, Hatch, Tobacman, & Craig, 2009). It is one of the myofibrillar proteins reported to be easily degraded by calpains during *post-mortem* aging of muscles. Indeed, Troponin T has been used as a marker of ongoing proteolysis (Iwanowska et al., 2010; Muroya et al., 2004) due to the appearance of polypeptides giving a product of 30 kDa and whose quantity progresses during the tenderization process and was found correlated to meat tenderness (Ho, Stromer, & Robson, 1994). As cited above in the sub-section 7.1.2, several proteomic studies reported TNNT fragments (fast and slow) as biomarkers of beef tenderness. This review highlights inverse relationships between LT and ST in agreement to studies from the large literature (de Oliveira, Delgado, Steadham, Huff-Lonergan, & Lonergan, 2019; Picard et al., 2018; Picard et al., 2014). Differences in line to this meta-proteomics among breeds or genders were further reported (Chaze et al., 2013; Rodrigues et al., 2017; Silva et al., 2019). For example, the recent study by Silva et al. (2019) found greater abundance of fast troponin T isoform (TNNT3) in steers LT muscle, while slow troponin T isoform (TNNT1) tended to be lower, compared to bulls.

7.3.3. PGM1, a biomarker of toughness in bulls and of tenderness in cows

Phosphoglucomutase 1 (PGM1) plays a central role in glucose homeostasis (Anderson, Lonergan, & Huff-Lonergan, 2012; Anderson et al., 2014) and its identification as a biomarker of beef tenderness is in agreement with the idea that in the early *post-mortem* period, glycolysis will continue, thereby affecting different quality traits of meat. PGM1 catalyzes the interconversion of glucose-1-phosphate and glucose-6-phosphate through the intermediate glucose-1,6-bisphosphate and thus plays an important role in the regulation of glycogen metabolism. Several posttranslational modifications (PTMs) through phosphorylation, acetylation and methylation of PGM1 were reported (Anderson et al., 2014). For example, 9 sites on PGM1 have been identified as phosphorylation sites and these include 2 serine, 3 tyrosine, and 4 threonine residues. PTMs were proposed to influence metabolism

and *post-mortem* muscle to meat conversion (Huang et al., 2011; Huang, Larsen, & Lametsch, 2012; Huang, Larsen, Palmisano, Dai, & Lametsch, 2014; Jiang, Liu, Shen, Zhou, & Shen, 2019; Li et al., 2015; Zhang et al., 2016). In fact, previous proteomic studies by D'Alessandro, Marrocco, et al. (2012) and (Silva et al., 2019) suggested that phosphorylation of muscle proteins, including PGM1, can be responsible for the differences in the final beef tenderness of steaks. Moreover, PGM1 is among the QTLs that were identified in this review whatever the muscle (**Figure 4**). We found this enzyme to be the lowest in young bulls (5 times) whatever the muscle and the highest in LT Charolais cows (1 time). Accordingly and in line to an earlier work, we reported that protein extracts from tender beef samples has less PGM1 compared to proteins extracted from tough beef steaks (Picard et al., 2010). It is worthwhile to note, that most proteomic studies identified multiple isoforms of PGM1 and their relationships with the targeted meat quality differ among isoforms according to the studied factors that are muscle, animal types, stress statue of the animal or response to postmortem electrical stimulation (Anderson et al., 2014; Bjarnadottir et al., 2010; D'Alessandro, Rinalducci, et al., 2012; Laville et al., 2009; Li et al., 2015).

7.3.4. TPI1 and YWHAE as biomarkers of tenderness whatever the muscle but with inverse relationships

Triosephosphate isomerase (TPI1) was identified only in Charolais breed, with lower abundance in tender LT of young bulls but not in ST and higher abundance in tender LT of steers and cows evaluated by WBSF. TPI1 is a glycolytic enzyme playing an important role in energy generation. It is the last enzyme of the first preparatory phase of glycolysis where it catalyzes the reversible conversion of D-glyceraldehyde 3-phosphate from phosphate dihydroxyacetone. Proteomic studies cited in sub-section 7.1.1 reported mainly positive and also negative associations with beef tenderness (D'Alessandro, Rinalducci, et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Grabez et al., 2015; Rosa et al., 2018). For example, it has been reported to be up regulated in LT tender meat of bulls by D'Alessandro, Marrocco, et al. (2012) and Grabez et al. (2015). *TPI1* gene was found to be significantly higher expressed in marbled cattle and proposed by several studies as a biomarker of intramuscular fat (Bazile, Picard, Chambon, Valais, & Bonnet, 2019; Ceciliani, Lecchi, Bazile, & Bonnet, 2018; N. K. Kim et al., 2009; Mao et al., 2016). From these, one can speculate that greater abundances in steers and cows known to be fatter than young bulls (Gagaoua, Terlouw, et al., 2016) could explain the inverse relationships with tenderness observed in the animal groups of this meta-proteomics. TPI1 including its phosphorylation

were further related with *post-mortem* muscle pH decline rate, comforting its role in meat tenderization (Gagaoua, Bonnet, De Koning, et al., 2018; Huang et al., 2011).

YWHAE was reported 4 times in this review and mostly with samples of LT and ST muscles of Charolais bulls and Steers evaluated by WBSF and also by sensory analysis in BA bulls (**Table 2**). The gene of this protein encodes tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, 14-3-3 epsilon. The 14-3-3 proteins (phospho-serine/-threonine binding proteins) belong to a large, highly conserved family functioning as a dimer in diverse biological processes such as signal transduction, metabolism, protein trafficking, signal transduction, apoptosis, cell cycle regulation and potassium channel regulation (Jin, Lyu, Kozak, & Jeang, 1996). This protein was reported as an interesting biomarker of beef tenderness in very recent studies (Chaze et al., 2013; D'Alessandro, Rinalducci, et al., 2012; Mahmood et al., 2018; Rodrigues et al., 2017). It was further reported by E. B. Carvalho et al. (2019) as a differential biomarker related to energy metabolism in skeletal muscle of beef cattle identified for low and high residual feed intake. This would explain as for TPI1, its link with tenderness of meat. Indeed, an earlier phosphoproteomic study reported that 14-3-3 proteins are able to regulate glucose homeostasis in response to insulin or to energetic stress (Ogihara et al., 1997). Furthermore, YWHAE is known to negatively regulate apoptosis (J. Kim et al., 2012), an important phase of muscle to meat conversion (Ouali et al., 2013; Ouali et al., 2006) with major consequences on final tenderness (Gagaoua, Hafid, et al., 2015). It was also reported to interact with HSPs such as HSPB1 as described in Picard et al. (2016). The results allowed to propose this protein as a candidate biomarker of mechanical tenderness in both LT and ST muscle mainly in males as it was higher abundant in tender ST and LT steers and lower abundant for LT young bulls, but not differential in cows.

7.3.5. Importance of structural MYH1, MYL1, MYL2 and MYBPH in beef tenderness

Myosin heavy, light and binding protein chains were identified as expected (Lana & Zolla, 2016; Mato et al., 2019) to play important role in the determinism of meat tenderness of young bulls and also of cows in both LT and ST muscle. Most of these proteins are grouped in the M-band which consists of a series of parallel electron dense lines in the central region of the A-band that comprises a filament system cross-linking the myosin filaments (For review: (Ertbjerg & Puolanne, 2017)). MYL1 was identified to be related positively only (3 times) in LT muscle of cows and ST of CH and BA of young bulls. Inversely, MYL2 that is also identified with a tenderness QTL, and negatively was less abundant in tender meat

whatever the evaluation method of tenderness and specific to young CH bulls (3 times) in LT and ST muscles and one time for ST muscle of LI bulls. MYBPH was less abundant in LT muscle of CH young bulls and cows, and highly abundant in ST muscle of LI and CH young bulls. Finally, MYH1 was negatively linked with tenderness in cows (LT muscle of CH and PDO Maine-Anjou cows) and positively in young bulls (ST and LT muscles of CH and BA young bulls). Myosins are the most abundant proteins of thick filaments and interaction of these structural proteins occur through the actomyosin complex that associates myosin light chains with myosin heavy chains and actin (Anderson et al., 2012). The identification of the 4 structural proteins as good biomarkers of beef tenderness agrees with several studies that we cited in sub-section 7.1.2 and further summarized in some recent comprehensive reviews (Ouali et al., 2013; Picard & Gagaoua, 2017; Picard et al., 2017). Among some studies, recent cattle proteomics on LT muscle showed similar findings with MYL1 that was reported in high abundance in less tender meat (Franco et al., 2015; Rodrigues et al., 2017; Rosa et al., 2018). In accordance to the findings of this meta-proteomics, Bjarnadottir et al. (2012) reported that MYL1 was more abundant in muscle biopsies from tender meat, while MYL2 was present in less abundance. The identification of MYL2 only in young bulls agrees with the findings by Rodrigues et al. (2017) showing increased muscle abundance in LT muscle of leaner breeds. Another work by Franco and co-workers identified that several muscle myosin light chains (MYL3 and MYL6B) and regulatory light chain 2 isoforms (MYL2 and MYLPF) participate in the conversion of cattle muscle to DFD meat in Rubia Gallega animals affected by pre-slaughter stress (Franco et al., 2015). In line to our findings namely the protein-protein networks of both muscles, the authors found also that all these structural proteins appeared to be involved in the main network of functionally associated proteins. Indeed, this is not a surprising finding when the myosin is a major structural protein of the muscle sarcomere in association with actin and other contractile proteins. Accordingly, all these structural proteins, well discussed in the comprehensive review by Lana and Zolla (2016), deserve further evaluation following the pipeline of biomarkers discovery and under other conditions to validate their robustness as biomarkers of beef tenderness.

7.4. Major beef tenderness biomarkers specific to LT muscle

7.4.1. HSPA1B, a robust candidate biomarker of WBSF whatever the gender

HSPA1B, a large HSP protein, for the three genders (young bulls, steers and cows) of different breeds (mostly CH and PDO Maine-Anjou) was less abundant in meat samples of

high tenderness evaluated by WBSF only,. This protein belongs to the most highly conserved 70 kDa HSPs family playing key roles as molecular chaperones and involved in promoting cellular proteostasis and survival during periods of stress (Mayer, 2013). They further regulate various cell signaling pathways involved in cell growth and inflammation (Daugaard, Rohde, & Jaattela, 2007). By interacting with other HSPs, HSPA1B protein stabilizes existing proteins against aggregation and mediates the folding of newly translated proteins in the cytosol and in organelles (M. E. Carvalho et al., 2014). It is particularly involved in the ubiquitin-proteasome pathway in accordance with the 2 proteasome subunits PSMB2, PSMC2 found differential between groups of tenderness only in LT muscle in the present review. These data suggest a role of ubiquitin-proteasome pathway in tenderness mainly in muscles with mixed oxido-glycolytic properties as LT. As cited above in sub-section 7.1.3 and not only for HSPA1B but also for the other 70 kDa HSPs proteins (HSPA1A, HSPA8 and HSPA5), dozen studies reported their involvement in meat tenderness under several factors such as breed (Chaze et al., 2013; Gagaoua, Terlouw, & Picard, 2017; Keady et al., 2013; Picard et al., 2014; Rodrigues et al., 2017), gender/animal type (Guillemin, Jurie, et al., 2011; Picard et al., 2019; Silva et al., 2019), rearing practices (Gagaoua, Monteils, et al., 2017), muscle type (Gagaoua, Couvreur, et al., 2017; Picard et al., 2014), evaluation method of tenderness (Gagaoua, Monteils, & Picard, 2018) and country origin of the panelists and end-point cooking temperature of meat (Gagaoua, Terlouw, et al., 2019). Recently, we proposed three main actions by which 70 kDa HSPs (HSPA1B) would intervene in *post-mortem* muscle and cooked meat, namely by **i)** responding to cellular stress since these proteins has been reported to be induced in skeletal muscle, **ii)** binding to the structural proteins to maintain homeostasis, or **iii)** protection roles of *post-mortem* muscle cells (Gagaoua, Terlouw, et al., 2019; Picard & Gagaoua, 2017).

7.4.2. ACTA1, a candidate biomarker specific to LT tenderness of young bulls and cows

ACTA1 or α-actin, a structural protein was related 5 times with tenderness in LT muscle only of young bulls whatever the breed (CH, LI and BA) and PDO Maine-Anjou cows. It was more abundant in the tender group samples and its direction varies in LI and CH breeds evaluated by WBSF and sensory LT of BA breed (**Table 2**). We previously stated that structural proteins, in general, are more related to tenderness in LT than in ST muscle, in coherence with the data of this review (Lana & Zolla, 2016; Picard & Gagaoua, 2017). According to Ouali et al. (2013), actin release from muscle cytoskeleton suggests apoptosis onset where caspases initiate with other interacting proteases the breakdown of actin into

fragments of different molecular weights (Goll, Neti, Mares, & Thompson, 2008; Ouali et al., 2013). ACTA1 is among the structural protein biomarkers of beef tenderness that were mostly identified in proteomic studies (Beldarrain et al., 2018; Bjarnadottir et al., 2012; Franco et al., 2015; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Lana & Zolla, 2016; Laville et al., 2009; Morzel et al., 2008; Picard & Gagaoua, 2017; Picard et al., 2017; Polati et al., 2012; Zapata et al., 2009). From these, ACTA1 deserves special attention as a robust biomarker of beef tenderness as it is further considered as a hallmark of apoptosis (Ouali et al., 2013). Indeed, during apoptosis, ACTA1 is the first protein targeted by effector caspases and some actin fragments have been considered to be accurate markers of apoptosis (Mashima, Naito, & Tsuruo, 1999; Yang et al., 1998). According to experiments by Du et al. (2004) and during apoptosis, the initial step in myofibrillar proteolysis would be the breakdown of the actomyosin complex by direct action of caspase 3. The authors pointed out that both intact monomeric actin and fragments of actin appear since early *post-mortem* including bovine muscle (Ouali et al., 2013). In a second step, these products will be then further degraded by the ATP-dependent ubiquitin-proteasome system, in line with the identification of the two proteasome subunits PSMB2 and PSMC2 found differential between groups of tenderness only in LT muscle of the same breeds where greater abundance of ACTA1 was found.

7.4.3. ENO1 and ENO3 highlight similarities between young bulls and cows

Enolase 1 (ENO1) and 3 (ENO3) are two isoforms catalyzing the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis. ENO1 is ubiquitous in cell cytoplasm, whereas ENO3 is mainly present in skeletal muscle. These two enzymes are predominately reported in proteomic studies to be related to beef tenderness and other qualities. From those studies and as highlighted in the sub-section 7.1.1, we report for ENO1 the studies by (D'Alessandro, Rinalducci, et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Polati et al., 2012) and for ENO3 those by (Bjarnadottir et al., 2012; Gagaoua, Bonnet, Ellies-Oury, et al., 2018; Marino et al., 2014; Polati et al., 2012; Zhao et al., 2014). In this meta-proteomics and in line with the findings of most of the above references, the two enolases were found 4 times. ENO1 was positively related with tenderness in LT muscle of cows (CH and PDO MA) and LI young bulls but negatively for CH young bulls. ENO3 was as for the former positively related with tenderness for cows whatever the breed (CH and PDO MA) but negatively for young bulls whatever the breed (BA and LI). The link of these two enzymes with meat tenderness was reported to be isoform specific (Picard & Gagaoua, 2017; Picard et al., 2017). The associations of tenderness with enolases may partly reflect a

cellular stress response to the deprivation of oxygen supply and to glucose metabolism (low glucose levels) (Sedoris, Thomas, & Miller, 2010). Indeed, these last authors reported that ENO3 expression is influenced by differences in glucose levels and deprivation of oxygen supply. It is worthwhile to note that ENO3 participates in multi-enzyme complexes present on the sarcomere (Keller et al., 2000) and their role in the tenderization process may further depend on the presence of other proteins identified in this meta-proteomics such as HSPs (Wulff, Jokumsen, Højrup, & Jessen, 2012).

7.4.4. HSPA9 and MSRA highlight similarities between steers and cows

HSPA9 (Stress-70 protein, mitochondrial GRP75) and MSRA (Mitochondrial peptide methionine sulfoxide reductase) were identified only in LT muscle and were specific to steers and cows (**Table 2**). The former was negatively related with tenderness and further reported one time and positively with sensory LT of young Limousin bulls. The later was positively related to tenderness of CH steers and cows and PDO Maine-Anjou cows.

HSPA9 is a member of Hsp70 family of chaperone proteins that is not heat-inducible. HSPA9 identified also as a tenderness QTL is known as mitochondrial HSP70, GRP75 or mortalin, that is predominantly localized in mitochondria but also in other cellular compartments such as endoplasmic reticulum, plasma membrane and cytoplasm (Kaul, Deocaris, & Wadhwa, 2007; Liu, Liu, Song, & Zuo, 2005). It is described as a multifunctional DnaK-type chaperone involved in many biological processes such as transportation of nucleus-encoded proteins to the mitochondrial matrix, cell protection against oxidative stress and apoptosis, import and translocation of cytosolic proteins, control of cell proliferation, muscle activity and proteasomal degradation of proteins (Kaul et al., 2007). This protein is the only chaperone described to be regulated by glucose privation, Ca^{2+} homeostasis and perturbation of glycolysis (Mayer, 2013). Accordingly, we previously found HSPA9 to be related to pH decline with strong correlation with pH_{3h} and ultimate pH (Gagaoua, Terlouw, Micol, et al., 2015). Numerous proteomic studies identified this protein as a good biomarker of beef tenderness (Grabez et al., 2015; Jia et al., 2009; Rodrigues et al., 2017). HSPA9 is able to enhance Ca^{2+} trafficking from endoplasmic reticulum toward mitochondria and cytosol (Glancy & Balaban, 2012).

In previous studies in cattle where this protein was identified as an interesting biomarker of beef qualities (Gagaoua, Couvreur, et al., 2017; Gagaoua, Terlouw, Boudjellal, et al., 2015; Gagaoua, Terlouw, Micol, et al., 2015; Gagaoua, Terlouw, & Picard, 2017), we suggested that

increased Ca^{2+} levels may exert regulatory effects on many proteolytic systems, namely, μ-calpain and proteins involved in metabolic and apoptotic processes. Accordingly, a biological mechanism involving HSPA9 in muscle to meat conversion was described (Picard & Gagaoua, 2017). The differential abundance of HSPA9 between groups of tenderness only in cows and steers and in LT muscle could mean that the protection against apoptosis and oxidative stress is higher in oxidative than in glycolytic muscles. The same hypothesis could be proposed for PARK7, or DJ-1, identified as a candidate biomarker of tenderness (**Table 2**) only in LT muscle in coherence with an interaction between these two proteins (Gagaoua, Terlouw, Boudjellal, et al., 2015).

MSRA is one of two mammalian methionine sulfoxide reductase isoforms. It is ubiquitous and highly conserved with mitochondrial and cytosolic forms translated from a single gene with localization dependent on alternative translation initiation sites. It is involved in the repair of oxidatively damaged proteins to restore their biological activity. Its main function is to convert methionine sulfoxide formed after methionine oxidation due to exposure to reactive oxygen species (ROS), to reduced methionine (Bin, Huang, & Zhou, 2017). Therefore, the action of MSRA has many cellular and physiological consequences as protection of cells against oxidative damage (Kantorow, Lee, & Chauss, 2012). The normal distribution of MSRA in the mammalian cell is approximately three folds greater in the cytosol than in mitochondria, hence suggesting potential role as antioxidant repair enzyme but with important role in apoptosis (Hunnicut, Liu, Richardson, & Salmon, 2015).

This meta-proteomics is the first to identify MSRA as a tenderness biomarker, but it was recently reported for beef color (Joseph, Suman, Rentfrow, Li, & Beach, 2012; Wu et al., 2016). The role of MSRA in tenderness and other meat quality traits could be through its cellular response to oxidative stress induced by *post-mortem* physiological conditions. It is well documented that oxidative stress induces damage to cellular proteins compromising their functions and leading to cellular dysfunction and finally cell death (Lana & Zolla, 2015; Ouali et al., 2013; L. L. Wang et al., 2018; Xing, Gao, Tume, Zhou, & Xu, 2019). Some authors described associations between MSRA with lipids and lipid-binding proteins (Lim, Kim, & Levine, 2017; Y. Y. Xu et al., 2015). This could partly explain why MSRA was found to be positively related to tenderness only in steers and cows which are known to be fatter than young bulls (Gagaoua, Terlouw, et al., 2016). We could speculate that in muscles with high intramuscular fat content that are likely with greater oxidative metabolism, oxidative stress is high and proteins such as MSRA would play pivotal role.

7.5. Major beef tenderness biomarkers specific to ST muscle

PVALB or Parvalbumin, was found in this review (4 times) specifically related to tenderness in ST muscle and only in young bulls whatever the breed and evaluation method (**Table 2**). To our knowledge, this meta-proteomic study is the first to report PVALB as a biomarker of beef tenderness. It was further identified by the ProteQTL tool of ProteINSIDE (http://www.proteinside.org/) as tenderness QTL. In Charolais (in 2 experiments) and Limousin young bulls, PVALB was higher abundant in tender groups a PVALB is a low molecular mass (9 – 11 kDa) calcium binding protein that regulates muscle contraction and relaxation (Cai et al., 2001). Several studies showed that PVALB is located exclusively in the sarcoplasm of type II (fast-twitch) mammalian skeletal muscle fibers (Muntener, Kaser, Weber, & Berchtold, 1995). The basic mechanism underlying the contraction–relaxation cycle of vertebrate muscles is based on a Ca^{2+} exchange between the sarcoplasmic reticulum and the myofibrils. Relaxation is achieved by retrieval of Ca^{2+} from the myofibrils and transfer to the sarcoplasmic reticulum. For example, mice with knockout of PVALB cannot properly regulate their intracellular Ca^{2+} concentration and require longer time to attain peak-twitch tension than their littermates (Cai et al., 2001). Its affinity to Ca^{2+} is higher than troponin C, but less than the sarcoplasmic reticulum Ca^{2+} ATPase pumps. From these, the role of PVALB in tenderness could be explained by its role in muscle relaxation through calcium binding and reduction of intracellular Ca^{2+}. The fact that PVALB is expressed in fast fibers could explain why it discriminated groups of tenderness in ST muscle and not those of LT. It is well documented in cattle that ST contains a higher proportion of fast fibers than LT (Gagaoua, Terlouw, Boudjellal, et al., 2015). Moreover, muscles of the hardy Salers breed are characterized by higher proportions of slow oxidative fibers than the other CH, LI or BA breeds (Jurie et al., 2005), thereby, explaining why PVALB was not identified as a discriminating biomarker of Salers tenderness groups. On another hand, it has been reported that ST muscle from continental and French beef breeds are tenderer when they are likely fast glycolytic (Picard et al., 2014). This is totally coherent with a higher abundance of PVALB in this type of muscle.

8. Conclusion and future perspectives

This integromics work of comparative proteomic studies from one laboratory aimed to identify robust candidate biomarkers of beef tenderness. Combined with data about published tenderness QTL and proteomics studies from the large literature, the results of this review

allow selecting among the list of 61 potential candidates those, which are more frequently differential between groups of tenderness according to the evaluation method of tenderness, muscles differing in their contractile and metabolic properties and breeds for three genders (young bulls, steers and cows). This meta-proteomics highlighted the importance of myriad biological pathways, namely the involvement of heat shock proteins with both small and large HSPs such as HSPB1 and HSPB6 identified whatever the factors described above. On another hand, HSPA1B, was found as a robust candidate biomarker of WBSF whatever the gender. Structural proteins were as expected identified in several studies and the most interesting for validation seemed to be Troponin T Fast (TnnT3), MYH1, MYL1, MYL2 MYBPH and ACTA1. Among the metabolic enzymes, PGM1, was found as a biomarker of toughness in bulls and of tenderness in cows and ENO1 and ENO3 highlight similarities between young bulls and cows. Among the list of the biomaekers, some of them highlighted muscle specificities such as Parvalbumin (PVALB) foud a major beef tenderness biomarker specific to ST muscle. Taken all together, the selected candidates are proposed for a deep analysis in the step "qualification and verification" of the pipeline of biomarkers discovery (Rifai et al., 2006) in order to validate their relationships with tenderness on a large number of samples and using several proteomics based approaches.

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References

Allais, S., Journaux, L., Leveziel, H., Payet-Duprat, N., Raynaud, P., Hocquette, J. F., Lepetit, J., Rousset, S., Denoyelle, C., Bernard-Capel, C., & Renand, G. (2011). Effects of polymorphisms in the calpastatin and mu-calpain genes on meat tenderness in 3 French beef breeds. *J Anim Sci, 89* (1), 1-11.

Anderson, M. J., Lonergan, S. M., & Huff-Lonergan, E. (2012). Myosin light chain 1 release from myofibrillar fraction during postmortem aging is a potential indicator of proteolysis and tenderness of beef. *Meat Sci, 90* (2), 345-351.

Anderson, M. J., Lonergan, S. M., & Huff-Lonergan, E. (2014). Differences in phosphorylation of phosphoglucomutase 1 in beef steaks from the longissimus dorsi with high or low star probe values. *Meat Sci, 96* (1), 379-384.

Aviles, C., Vinet, A., Saintilan, R., Picard, B., Lepetit, J., Dumont, R., Oury, M.-P., Krauss, D., Maupetit, D., & Renand, G. (2014). Genetic correlations between carcass and meat quality traits of adult cows and similar traits of young Bulls in French Charolais beef cattle. In *10th World Congress on Genetics Applied to Livestock Production. 2014; 10. World Congress on Genetics Applied to Livestock Production (WCGALP), Vancouver, CAN, 2014-08-17-2014-08-22, 1-3*.

Baldassini, W. A., Braga, C. P., Chardulo, L. A., Vasconcelos Silva, J. A., 2nd, Malheiros, J. M., de Albuquerque, L. G., Fernandes, T. T., & Padilha Pde, M. (2015). Bioanalytical methods for the metalloproteomics study of bovine longissimus thoracis muscle tissue with different grades of meat tenderness in the Nellore breed (Bos indicus). *Food Chem, 169* (0), 65-72.

Bazile, J., Picard, B., Chambon, C., Valais, A., & Bonnet, M. (2019). Pathways and biomarkers of marbling and carcass fat deposition in bovine revealed by a combination of gel-based and gel-free proteomic analyses. *Meat Sci, 156*, 146-155.

Beldarrain, L. R., Aldai, N., Picard, B., Sentandreu, E., Navarro, J. L., & Sentandreu, M. A. (2018). Use of liquid isoelectric focusing (OFFGEL) on the discovery of meat tenderness biomarkers. *Journal of Proteomics, 183*, 25-33.

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E.-D. A. (2018). Applied and Emerging Methods for Meat Tenderization: A Comparative Perspective. *Comprehensive Reviews in Food Science and Food Safety, 17* (4), 841-859.

Bin, P., Huang, R., & Zhou, X. (2017). Oxidation Resistance of the Sulfur Amino Acids: Methionine and Cysteine. *Biomed Res Int, 2017*, 9584932.

Bjarnadottir, S. G., Hollung, K., Faergestad, E. M., & Veiseth-Kent, E. (2010). Proteome changes in bovine longissimus thoracis muscle during the first 48 h postmortem: shifts in energy status and myofibrillar stability. *J Agric Food Chem, 58* (12), 7408-7414.

Bjarnadottir, S. G., Hollung, K., Hoy, M., Bendixen, E., Codrea, M. C., & Veiseth-Kent, E. (2012). Changes in protein abundance between tender and tough meat from bovine longissimus thoracis muscle assessed by isobaric Tag for Relative and Absolute Quantitation (iTRAQ) and 2-dimensional gel electrophoresis analysis. *J Anim Sci, 90* (6), 2035-2043.

Bouley, J., Chambon, C., & Picard, B. (2004). Mapping of bovine skeletal muscle proteins using two-dimensional gel electrophoresis and mass spectrometry. *PROTEOMICS, 4* (6), 1811-1824.

Bouley, J., Meunier, B., Chambon, C., De Smet, S., Hocquette, J. F., & Picard, B. (2005). Proteomic analysis of bovine skeletal muscle hypertrophy. *PROTEOMICS, 5* (2), 490-500.

Cai, D. Q., Li, M., Lee, K. K., Lee, K. M., Qin, L., & Chan, K. M. (2001). Parvalbumin expression is downregulated in rat fast-twitch skeletal muscles during aging. *Arch Biochem Biophys, 387* (2), 202-208.

Carvalho, E. B., Gionbelli, M. P., Rodrigues, R. T. S., Bonilha, S. F. M., Newbold, C. J., Guimaraes, S. E. F., Silva, W., Verardo, L. L., Silva, F. F., Detmann, E., & Duarte, M. S. (2019). Differentially expressed mRNAs, proteins and miRNAs associated to energy metabolism in skeletal muscle of beef cattle identified for low and high residual feed intake. *BMC Genomics, 20* (1), 501.

Carvalho, M. E., Gasparin, G., Poleti, M. D., Rosa, A. F., Balieiro, J. C., Labate, C. A., Nassu, R. T., Tullio, R. R., Regitano, L. C., Mourao, G. B., & Coutinho, L. L. (2014). Heat shock and structural proteins associated with meat tenderness in Nellore beef cattle, a Bos indicus breed. *Meat Sci, 96* (3), 1318-1324.

Ceciliani, F., Lecchi, C., Bazile, J., & Bonnet, M. (2018). Proteomics Research in the Adipose Tissue. In A. M. de Almeida, D. Eckersall & I. Miller (Eds.), *Proteomics in Domestic Animals: from Farm to Systems Biology* (pp. 233-254). Cham: Springer International Publishing.

Chaze, T., Hocquette, J. F., Meunier, B., Renand, G., Journaux, L., Capel, C., & Picard, B. (2009). Research of beef tenderness markers in young bulls by proteomic analysis. *16 èmes Rencontres autour des Recherches sur les Ruminants, Paris, les 2 et 3 Décembre 2009*, 151-154.

Chaze, T., Hocquette, J. F., Meunier, B., Renand, G., Jurie, C., Chambon, C., Journaux, L., Rousset, L., Denoyelle, C., Lepetit, J., & Picard, B. (2013). Biological Markers for Meat Tenderness of the Three Main French Beef Breeds Using 2-DE and MS Approach. In F. Toldrá & L. M. L. Nollet (Eds.), *Proteomics in Foods* (Vol. 2, pp. 127-146): Springer US.

Christensen, R. G., Ineck, N. E., Phelps, K. J., Ebarb, S. M., Drouillard, J. S., Gonzalez, J. M., & Thornton, K. J. (2017). Small Heat Shock Protein Expression Differs During Aging

in Steaks from the Longissimus Lumborum of Cattle that Received Different Growth Promotants. *Meat and Muscle Biology, 1* (3), 147-147.

Contreras-Castillo, C. J., Lomiwes, D., Wu, G., Frost, D., & Farouk, M. M. (2015). The effect of electrical stimulation on post mortem myofibrillar protein degradation and small heat shock protein kinetics in bull beef. *Meat Sci*.

Couvreur, S., Le Bec, G., Micol, D., & Picard, B. (2019). Relationships Between Cull Beef Cow Characteristics, Finishing Practices and Meat Quality Traits of Longissimus thoracis and Rectus abdominis. *Foods, 8* (4).

D'Alessandro, A., Marrocco, C., Rinalducci, S., Mirasole, C., Failla, S., & Zolla, L. (2012). Chianina beef tenderness investigated through integrated Omics. *J Proteomics, 75* (14), 4381-4398.

D'Alessandro, A., Rinalducci, S., Marrocco, C., Zolla, V., Napolitano, F., & Zolla, L. (2012). Love me tender: an Omics window on the bovine meat tenderness network. *J Proteomics, 75* (14), 4360-4380.

D'Alessandro, A., & Zolla, L. (2013). Meat science: From proteomics to integrated omics towards system biology. *J Proteomics, 78* (0), 558-577.

Daugaard, M., Rohde, M., & Jaattela, M. (2007). The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. *FEBS Lett, 581* (19), 3702-3710.

de Oliveira, L. G., Delgado, E. F., Steadham, E. M., Huff-Lonergan, E., & Lonergan, S. M. (2019). Association of calpain and calpastatin activity to postmortem myofibrillar protein degradation and sarcoplasmic proteome changes in bovine Longissiumus lumborum and Triceps brachii. *Meat Sci, 155*, 50-60.

Dransfield, E., Martin, J.-F., Bauchart, D., Abouelkaram, S., Lepetit, J., Culioli, J., Jurie, C., & Picard, B. (2003). Meat quality and composition of three muscles from French cull cows and young bulls. *Animal Science, 76*, 387-399.

Dreiza, C. M., Komalavilas, P., Furnish, E. J., Flynn, C. R., Sheller, M. R., Smoke, C. C., Lopes, L. B., & Brophy, C. M. (2010). The small heat shock protein, HSPB6, in muscle function and disease. *Cell Stress Chaperones, 15* (1), 1-11.

Du, J., Wang, X., Miereles, C., Bailey, J. L., Debigare, R., Zheng, B., Price, S. R., & Mitch, W. E. (2004). Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *J Clin Invest, 113* (1), 115-123.

Enns, D. L., & Tiidus, P. M. (2010). The Influence of Estrogen on Skeletal Muscle. *Sports Medicine, 40* (1), 41-58.

Ertbjerg, P., & Puolanne, E. (2017). Muscle structure, sarcomere length and influences on meat quality: A review. *Meat Sci, 132*, 139-152.

Fink, S., Momke, S., Wohlke, A., & Distl, O. (2008). Genes on bovine chromosome 18 associated with bilateral convergent strabismus with exophthalmos in German Brown cattle. *Mol Vis, 14*, 1737-1751.

Franco, D., Mato, A., Salgado, F. J., Lopez-Pedrouso, M., Carrera, M., Bravo, S., Parrado, M., Gallardo, J. M., & Zapata, C. (2015). Tackling proteome changes in the longissimus thoracis bovine muscle in response to pre-slaughter stress. *J Proteomics, 122* (0), 73-85.

Gagaoua, M., Bonnet, M., De Koning, L., & Picard, B. (2018). Reverse Phase Protein array for the quantification and validation of protein biomarkers of beef qualities: The case of meat color from Charolais breed. *Meat Sci, 145*, 308-319.

Gagaoua, M., Bonnet, M., Ellies-Oury, M. P., De Koning, L., & Picard, B. (2018). Reverse phase protein arrays for the identification/validation of biomarkers of beef texture and their use for early classification of carcasses. *Food Chemistry, 250* (C), 245-252.

Gagaoua, M., Couvreur, S., Le Bec, G., Aminot, G., & Picard, B. (2017). Associations among Protein Biomarkers and pH and Color Traits in Longissimus thoracis and Rectus abdominis Muscles in Protected Designation of Origin Maine-Anjou Cull Cows. *J Agric Food Chem, 65* (17), 3569-3580.

Gagaoua, M., Hafid, K., Boudida, Y., Becila, S., Ouali, A., Picard, B., Boudjellal, A., & Sentandreu, M. A. (2015). Caspases and Thrombin Activity Regulation by Specific Serpin Inhibitors in Bovine Skeletal Muscle. *Appl Biochem Biotechnol, 177* (2), 279-303.

Gagaoua, M., Micol, D., Picard, B., Terlouw, C. E., Moloney, A. P., Juin, H., Meteau, K., Scollan, N., Richardson, I., & Hocquette, J. F. (2016). Inter-laboratory assessment by trained panelists from France and the United Kingdom of beef cooked at two different end-point temperatures. *Meat Sci, 122*, 90-96.

Gagaoua, M., Monteils, V., Couvreur, S., & Picard, B. (2017). Identification of Biomarkers Associated with the Rearing Practices, Carcass Characteristics, and Beef Quality: An Integrative Approach. *Journal of Agricultural and Food Chemistry, 65* (37), 8264-8278.

Gagaoua, M., Monteils, V., Couvreur, S., & Picard, B. (2019). Beef Tenderness Prediction by a Combination of Statistical Methods: Chemometrics and Supervised Learning to Manage Integrative Farm-To-Meat Continuum Data. *Foods, 8* (7), 274.

Gagaoua, M., Monteils, V., & Picard, B. (2018). Data from the farmgate-to-meat continuum including omics-based biomarkers to better understand the variability of beef tenderness: An integromics approach. *J Agric Food Chem, 66* (51), 13552–13563.

Gagaoua, M., Monteils, V., & Picard, B. (2019). Decision tree, a learning tool for the prediction of beef tenderness using rearing factors and carcass characteristics. *J Sci Food Agric, 99* (3), 1275-1283.

Gagaoua, M., Picard, B., & Monteils, V. (2019). Assessment of cattle inter-individual cluster variability: the potential of continuum data from the farm-to-fork for ultimate beef tenderness management. *J Sci Food Agric, 99* (8), 4129-4141.

Gagaoua, M., Picard, B., Soulat, J., & Monteils, V. (2018). Clustering of sensory eating qualities of beef: Consistencies and differences within carcass, muscle, animal characteristics and rearing factors. *Livestock Science, 214*, 245-258.

Gagaoua, M., Terlouw, C., Richardson, I., Hocquette, J. F., & Picard, B. (2019). The associations between proteomic biomarkers and beef tenderness depend on the end-point cooking temperature, the country origin of the panelists and breed. *Meat Sci, 157* (C), 107871.

Gagaoua, M., Terlouw, E. M., Boudjellal, A., & Picard, B. (2015). Coherent correlation networks among protein biomarkers of beef tenderness: What they reveal. *J Proteomics, 128*, 365-374.

Gagaoua, M., Terlouw, E. M., Micol, D., Boudjellal, A., Hocquette, J. F., & Picard, B. (2015). Understanding Early Post-Mortem Biochemical Processes Underlying Meat Color and pH Decline in the Longissimus thoracis Muscle of Young Blond d'Aquitaine Bulls Using Protein Biomarkers. *J Agric Food Chem, 63* (30), 6799-6809.

Gagaoua, M., Terlouw, E. M. C., Micol, D., Hocquette, J. F., Moloney, A. P., Nuernberg, K., Bauchart, D., Boudjellal, A., Scollan, N. D., Richardson, R. I., & Picard, B. (2016). Sensory quality of meat from eight different types of cattle in relation with their biochemical characteristics. *Journal of Integrative Agriculture, 15* (7), 1550-1563.

Gagaoua, M., Terlouw, E. M. C., Monteils, V., Couvreur, S., & Picard, B. (2017). Stress proteins in cull cows: relationships with transport and lairage durations but not with meat tenderness. In D. Troy, C. McDonnell, L. Hinds & J. Kerry (Eds.), *Proceedings of the 63rd International Congress of Meat Science and Technology* (First edition ed., pp. 427-428). Cork, Ireland: Wageningen Academic Publishers.

Gagaoua, M., Terlouw, E. M. C., & Picard, B. (2017). The study of protein biomarkers to understand the biochemical processes underlying beef color development in young bulls. *Meat Sci, 134*, 18-27.

Ghosh, J. G., Houck, S. A., & Clark, J. I. (2007). Interactive sequences in the stress protein and molecular chaperone human αB crystallin recognize and modulate the assembly of filaments. *The International Journal of Biochemistry & Cell Biology, 39* (10), 1804-1815.

Glancy, B., & Balaban, R. S. (2012). Role of mitochondrial Ca2+ in the regulation of cellular energetics. *Biochemistry, 51* (14), 2959-2973.

Golenhofen, N., Perng, M. D., Quinlan, R. A., & Drenckhahn, D. (2004). Comparison of the small heat shock proteins alphaB-crystallin, MKBP, HSP25, HSP20, and cvHSP in heart and skeletal muscle. *Histochem Cell Biol, 122* (5), 415-425.

Goll, D., Neti, G., Mares, S., & Thompson, V. (2008). Myofibrillar protein turnover: the proteasome and the calpains. *Journal of Animal Science, 86* (14_suppl), E19-E35.

Görg, A., Weiss, W., & Dunn, M. J. (2004). Current two-dimensional electrophoresis technology for proteomics. *PROTEOMICS, 4* (12), 3665-3685.

Grabez, V., Kathri, M., Phung, V., Moe, K. M., Slinde, E., Skaugen, M., Saarem, K., & Egelandsdal, B. (2015). Protein expression and oxygen consumption rate of early postmortem mitochondria relate to meat tenderness. *J Anim Sci, 93* (4), 1967-1979.

Grunert, K. G., Bredahl, L., & Brunsø, K. (2004). Consumer perception of meat quality and implications for product development in the meat sector—a review. *Meat Sci, 66* (2), 259-272.

Guillemin, N., Bonnet, M., Jurie, C., & Picard, B. (2011). Functional analysis of beef tenderness. *J Proteomics, 75* (2), 352-365.

Guillemin, N., Jurie, C., Cassar-Malek, I., Hocquette, J. F., Renand, G., & Picard, B. (2011). Variations in the abundance of 24 protein biomarkers of beef tenderness according to muscle and animal type. *Animal, 5* (6), 885-894.

Guillemin, N., Meunier, B., Jurie, C., Cassar-Malek, I., Hocquette, J. F., Leveziel, H., & Picard, B. (2009). Validation of a Dot-Blot quantitative technique for large scale analysis of beef tenderness biomarkers. *J Physiol Pharmacol, 60 Suppl 3*, 91-97.

Hao, Y., Feng, Y., Yang, P., Cui, Y., Liu, J., Yang, C., & Gu, X. (2016). Transcriptome analysis reveals that constant heat stress modifies the metabolism and structure of the porcine longissimus dorsi skeletal muscle. *Molecular Genetics and Genomics, 291* (6), 2101-2115.

Ho, C. Y., Stromer, M. H., & Robson, R. M. (1994). Identification of the 30 kDa polypeptide in post mortem skeletal muscle as a degradation product of troponin-T. *Biochimie, 76* (5), 369-375.

Hu, Z. L., Park, C. A., Wu, X. L., & Reecy, J. M. (2013). Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era. *Nucleic Acids Res, 41* (Database issue), D871-879.

Huang, H., Larsen, M. R., Karlsson, A. H., Pomponio, L., Costa, L. N., & Lametsch, R. (2011). Gel-based phosphoproteomics analysis of sarcoplasmic proteins in postmortem porcine muscle with pH decline rate and time differences. *PROTEOMICS, 11* (20), 4063-4076.

Huang, H., Larsen, M. R., & Lametsch, R. (2012). Changes in phosphorylation of myofibrillar proteins during postmortem development of porcine muscle. *Food Chem, 134* (4), 1999-2006.

Huang, H., Larsen, M. R., Palmisano, G., Dai, J., & Lametsch, R. (2014). Quantitative phosphoproteomic analysis of porcine muscle within 24h postmortem. *J Proteomics, 106* (0), 125-139.

Hunnicut, J., Liu, Y., Richardson, A., & Salmon, A. B. (2015). MsrA Overexpression Targeted to the Mitochondria, but Not Cytosol, Preserves Insulin Sensitivity in Diet-Induced Obese Mice. *PLOS ONE, 10* (10), e0139844.

Hwang, J. H., Kwon, S. G., Park, D. H., Kim, T. W., Kang, D. G., Ha, J., Kim, S. W., & Kim, C. W. (2015). Molecular characterization of porcine PGM1 gene associated with meat quality traits. *Canadian Journal of Animal Science, 95* (1), 31-36.

Iwanowska, A., Iwańska, E., Grześ, B., Mikołajczak, B., Pospiech, E., Rosochacki, S., Juszczuk–Kubiak, E., & Łyczyński, A. (2010). Changes in proteins and tenderness of

meat from young bulls of four breeds at three ages over 10 days of cold storage. *Animal Science Papers and Reports, 28* (1), 13-25.

Jia, X., Ekman, M., Grove, H., Faergestad, E. M., Aass, L., Hildrum, K. I., & Hollung, K. (2007). Proteome changes in bovine longissimus thoracis muscle during the early postmortem storage period. *J Proteome Res, 6* (7), 2720-2731.

Jia, X., Hildrum, K. I., Westad, F., Kummen, E., Aass, L., & Hollung, K. (2006). Changes in enzymes associated with energy metabolism during the early post mortem period in longissimus thoracis bovine muscle analyzed by proteomics. *J Proteome Res, 5* (7), 1763-1769.

Jia, X., Veiseth-Kent, E., Grove, H., Kuziora, P., Aass, L., Hildrum, K. I., & Hollung, K. (2009). Peroxiredoxin-6--a potential protein marker for meat tenderness in bovine longissimus thoracis muscle. *J Anim Sci, 87* (7), 2391-2399.

Jiang, S., Liu, Y., Shen, Z., Zhou, B., & Shen, Q. W. (2019). Acetylome profiling reveals extensive involvement of lysine acetylation in the conversion of muscle to meat. *J Proteomics, 205*, 103412.

Jin, D. Y., Lyu, M. S., Kozak, C. A., & Jeang, K. T. (1996). Function of 14-3-3 proteins. *Nature, 382* (6589), 308.

Joseph, P., Suman, S. P., Rentfrow, G., Li, S., & Beach, C. M. (2012). Proteomics of muscle-specific beef color stability. *J Agric Food Chem, 60* (12), 3196-3203.

Jurie, C., Martin, J.-F., Listrat, A., Jailler, R., Culioli, J., & Picard, B. (2005). Effects of age and breed of beef bulls on growth parameters, carcass and muscle characteristics. *Animal Science, 80* (03), 257-263.

Jurinke, C., Oeth, P., & van den Boom, D. (2004). MALDI-TOF mass spectrometry. *Molecular Biotechnology, 26* (2), 147-163.

Kammoun, M., Picard, B., Astruc, T., Gagaoua, M., Aubert, D., Bonnet, M., Blanquet, V., & Cassar-Malek, I. (2016). The Invalidation of *HspB1* Gene in Mouse Alters the Ultrastructural Phenotype of Muscles. *PLOS ONE, 11* (8), e0158644.

Kantorow, M., Lee, W., & Chauss, D. (2012). Focus on Molecules: methionine sulfoxide reductase A. *Exp Eye Res, 100*, 110-111.

Kaspric, N., Picard, B., Reichstadt, M., Tournayre, J., & Bonnet, M. (2015). ProteINSIDE to Easily Investigate Proteomics Data from Ruminants: Application to Mine Proteome of Adipose and Muscle Tissues in Bovine Foetuses. *PLOS ONE, 10* (5), e0128086.

Kaul, S. C., Deocaris, C. C., & Wadhwa, R. (2007). Three faces of mortalin: a housekeeper, guardian and killer. *Exp Gerontol, 42* (4), 263-274.

Keady, S. M., Kenny, D. A., Ohlendieck, K., Doyle, S., Keane, M. G., & Waters, S. M. (2013). Proteomic profiling of bovine M. longissimus lumborum from Crossbred Aberdeen Angus and Belgian Blue sired steers varying in genetic merit for carcass weight. *J Anim Sci, 91* (2), 654-665.

Keller, A., Demeurie, J., Merkulova, T., Géraud, G., Cywiner-Golenzer, C., Lucas, M., & Châtelet, F.-P. (2000). Fibre-type distribution and subcellular localisation of α and β enolase in mouse striated muscle. *Biology of the Cell, 92* (7), 527-535.

Kim, J., Parrish, A. B., Kurokawa, M., Matsuura, K., Freel, C. D., Andersen, J. L., Johnson, C. E., & Kornbluth, S. (2012). Rsk-mediated phosphorylation and 14-3-3varepsilon binding of Apaf-1 suppresses cytochrome c-induced apoptosis. *EMBO J, 31* (5), 1279-1292.

Kim, N. K., Cho, S., Lee, S. H., Park, H. R., Lee, C. S., Cho, Y. M., Choy, Y. H., Yoon, D., Im, S. K., & Park, E. W. (2008). Proteins in longissimus muscle of Korean native cattle and their relationship to meat quality. *Meat Sci, 80* (4), 1068-1073.

Kim, N. K., Lee, S. H., Cho, Y. M., Son, E. S., Kim, K. Y., Lee, C. S., Yoon, D., Im, S. K., Oh, S. J., & Park, E. W. (2009). Proteome analysis of the m. longissimus dorsi between fattening stages in Hanwoo steer. *BMB Rep, 42* (7), 433-438.

Kim, Y. H. B., Warner, R. D., & Rosenvold, K. (2014). Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. *Animal Production Science, 54* (4), 375-395.

Kraus, V. B., Burnett, B., Coindreau, J., Cottrell, S., Eyre, D., Gendreau, M., Gardiner, J., Garnero, P., Hardin, J., Henrotin, Y., Heinegard, D., Ko, A., Lohmander, L. S., Matthews, G., Menetski, J., Moskowitz, R., Persiani, S., Poole, A. R., Rousseau, J. C., Todman, M., & Group, O. F. O. B. W. (2011). Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. *Osteoarthritis Cartilage, 19* (5), 515-542.

Lana, A., & Zolla, L. (2015). Apoptosis or autophagy, that is the question: Two ways for muscle sacrifice towards meat. *Trends in Food Science & Technology, 46* (2), 231-241.

Lana, A., & Zolla, L. (2016). Proteolysis in meat tenderization from the point of view of each single protein: A proteomic perspective. *Journal of Proteomics, 147*, 85-97.

Laville, E., Sayd, T., Morzel, M., Blinet, S., Chambon, C., Lepetit, J., Renand, G., & Hocquette, J. F. (2009). Proteome changes during meat aging in tough and tender beef suggest the importance of apoptosis and protein solubility for beef aging and tenderization. *J Agric Food Chem, 57* (22), 10755-10764.

Lehman, W., Galińska-Rakoczy, A., Hatch, V., Tobacman, L. S., & Craig, R. (2009). Structural Basis for the Activation of Muscle Contraction by Troponin and Tropomyosin. *Journal of Molecular Biology, 388* (4), 673-681.

Li, C., Zhou, G., Xu, X., Lundstrom, K., Karlsson, A., & Lametsch, R. (2015). Phosphoproteome analysis of sarcoplasmic and myofibrillar proteins in bovine longissimus muscle in response to postmortem electrical stimulation. *Food Chem, 175* (0), 197-202.

Lim, J. M., Kim, G., & Levine, R. L. (2017). Myristoylated Methionine Sulfoxide Reductase A Interacts with the Lipid-Binding Protein. *Free Radical Biology and Medicine, 112*, 28-29.

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B., & Bugeon, J. (2016). How Muscle Structure and Composition Influence Meat and Flesh Quality. *ScientificWorldJournal, 2016*, 3182746.

Liu, Y., Liu, W., Song, X. D., & Zuo, J. (2005). Effect of GRP75/mthsp70/PBP74/mortalin overexpression on intracellular ATP level, mitochondrial membrane potential and ROS accumulation following glucose deprivation in PC12 cells. *Mol Cell Biochem, 268* (1-2), 45-51.

Lomiwes, D., Farouk, M. M., Frost, D. A., Dobbie, P. M., & Young, O. A. (2013). Small heat shock proteins and toughness in intermediate pHu beef. *Meat Sci, 95* (3), 472-479.

Lomiwes, D., Farouk, M. M., Wiklund, E., & Young, O. A. (2014). Small heat shock proteins and their role in meat tenderness: a review. *Meat Sci, 96* (1), 26-40.

Longo, V., Lana, A., Bottero, M. T., & Zolla, L. (2015). Apoptosis in muscle-to-meat aging process: The omic witness. *J Proteomics, 125* (0), 29-40.

López-Pedrouso, M., Bernal, J., Franco, D., & Zapata, C. (2014). Evaluating Two-Dimensional Electrophoresis Profiles of the Protein Phaseolin as Markers of Genetic Differentiation and Seed Protein Quality in Common Bean (Phaseolus vulgaris L.). *Journal of Agricultural and Food Chemistry, 62* (29), 7200-7208.

Lopez-Pedrouso, M., Franco, D., Serrano, M. P., Maggiolino, A., Landete-Castillejos, T., De Palo, P., & Lorenzo, J. M. (2019). A proteomic-based approach for the search of biomarkers in Iberian wild deer (Cervus elaphus) as indicators of meat quality. *J Proteomics, 205*, 103422.

Mahmood, S., Turchinsky, N., Paradis, F., Dixon, W. T., & Bruce, H. L. (2018). Proteomics of dark cutting longissimus thoracis muscle from heifer and steer carcasses. *Meat Sci, 137*, 47-57.

Malheiros, J. M., Braga, C. P., Grove, R. A., Ribeiro, F. A., Calkins, C. R., Adamec, J., & Chardulo, L. A. L. (2019). Influence of oxidative damage to proteins on meat tenderness using a proteomics approach. *Meat Sci, 148*, 64-71.

Mao, Y., Hopkins, D. L., Zhang, Y., Li, P., Zhu, L., Dong, P., Liang, R., Dai, J., Wang, X., & Luo, X. (2016). Beef quality with different intramuscular fat content and proteomic analysis using isobaric tag for relative and absolute quantitation of differentially expressed proteins. *Meat Sci, 118*, 96-102.

Marino, R., Albenzio, M., Della Malva, A., Caroprese, M., Santillo, A., & Sevi, A. (2014). Changes in meat quality traits and sarcoplasmic proteins during aging in three different cattle breeds. *Meat Sci, 98* (2), 178-186.

Mashima, T., Naito, M., & Tsuruo, T. (1999). Caspase-mediated cleavage of cytoskeletal actin plays a positive role in the process of morphological apoptosis. *Oncogene, 18* (15), 2423-2430.

Mato, A., Rodríguez-Vázquez, R., López-Pedrouso, M., Bravo, S., Franco, D., & Zapata, C. (2019). The first evidence of global meat phosphoproteome changes in response to pre-slaughter stress. *BMC Genomics, 20* (1), 590.

Mayer, M. P. (2013). Hsp70 chaperone dynamics and molecular mechanism. *Trends Biochem Sci, 38* (10), 507-514.

Meunier, B., Bouley, J., Piec, I., Bernard, C., Picard, B., & Hocquette, J.-F. (2005). Data analysis methods for detection of differential protein expression in two-dimensional gel electrophoresis. *Analytical Biochemistry, 340* (2), 226-230.

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *J Anim Sci, 79* (12), 3062-3068.

Morzel, M., Terlouw, C., Chambon, C., Micol, D., & Picard, B. (2008). Muscle proteome and meat eating qualities of Longissimus thoracis of "Blonde d'Aquitaine" young bulls: A central role of HSP27 isoforms. *Meat Sci, 78* (3), 297-304.

Mullen, A. M., Stapleton, P. C., Corcoran, D., Hamill, R. M., & White, A. (2006). Understanding meat quality through the application of genomic and proteomic approaches. *Meat Sci, 74* (1), 3-16.

Muntener, M., Kaser, L., Weber, J., & Berchtold, M. W. (1995). Increase of skeletal muscle relaxation speed by direct injection of parvalbumin cDNA. *Proc Natl Acad Sci U S A, 92* (14), 6504-6508.

Muroya, S., Kitamura, S., Tanabe, S., Nishimura, T., Nakajima, I., & Chikuni, K. (2004). N-terminal amino acid sequences of troponin T fragments, including 30 kDa one, produced during postmortem aging of bovine longissimus muscle. *Meat Sci, 67* (1), 19-24.

Ogihara, T., Isobe, T., Ichimura, T., Taoka, M., Funaki, M., Sakoda, H., Onishi, Y., Inukai, K., Anai, M., Fukushima, Y., Kikuchi, M., Yazaki, Y., Oka, Y., & Asano, T. (1997). 14-3-3 protein binds to insulin receptor substrate-1, one of the binding sites of which is in the phosphotyrosine binding domain. *J Biol Chem, 272* (40), 25267-25274.

Ouali, A., Gagaoua, M., Boudida, Y., Becila, S., Boudjellal, A., Herrera-Mendez, C. H., & Sentandreu, M. A. (2013). Biomarkers of meat tenderness: present knowledge and perspectives in regards to our current understanding of the mechanisms involved. *Meat Sci, 95* (4), 854-870.

Ouali, A., Herrera-Mendez, C. H., Coulis, G., Becila, S., Boudjellal, A., Aubry, L., & Sentandreu, M. A. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. *Meat Sci, 74* (1), 44-58.

Ouali, A., Sentandreu, M. A., Aubry, L., Boudjellal, A., Tassy, C., Geesink, G. H., & Faris-Maffet, G. (2005). Meat toughness as affected by muscle type. In J. F. Hocquette & S. Gigli (Eds.), *Indicators of milk and beef quality* (Vol. 112, pp. 391-395). Wageningen, The Netherlands: Wageningen Academic Publishers.

Picard, B., Berri, C., Lefaucheur, L., Molette, C., Sayd, T., & Terlouw, C. (2010). Skeletal muscle proteomics in livestock production. *Brief Funct Genomics, 9* (3), 259-278.

Picard, B., Chambon, C., Meunier, B., Jacob, H., & Jurie, C. (2006). Marqueurs de tendrete de la viande de taurillons blonde d'aquitaine reveles par l'analyse du proteome. *Viandes et produits carnés*, 139-140.

Picard, B., & Gagaoua, M. (2017). Chapter 11 - Proteomic Investigations of Beef Tenderness. In M. L. Colgrave (Ed.), *Proteomics in Food Science: from farm to fork* (pp. 177-197). London: Academic Press.

Picard, B., Gagaoua, M., Al-Jammas, M., De Koning, L., Valais, A., & Bonnet, M. (2018). Beef tenderness and intramuscular fat proteomic biomarkers: muscle type effect. *PeerJ, 6*, e4891.

Picard, B., Gagaoua, M., Al Jammas, M., & Bonnet, M. (2019). Beef tenderness and intramuscular fat proteomic biomarkers: Effect of gender and rearing practices. *J Proteomics, 200*, 1-10.

Picard, B., Gagaoua, M., & Hollung, K. (2017). Chapter 12 - Gene and Protein Expression as a Tool to Explain/Predict Meat (and Fish) Quality In P. Purslow (Ed.), *New Aspects of Meat Quality : From Genes to Ethics* (pp. 321-354). United Kingdom: Woodhead Publishing.

Picard, B., Gagaoua, M., Micol, D., Cassar-Malek, I., Hocquette, J. F., & Terlouw, C. E. (2014). Inverse relationships between biomarkers and beef tenderness according to contractile and metabolic properties of the muscle. *J Agric Food Chem, 62* (40), 9808-9818.

Picard, B., Kammoun, M., Gagaoua, M., Barboiron, C., Meunier, B., Chambon, C., & Cassar-Malek, I. (2016). Calcium Homeostasis and Muscle Energy Metabolism Are Modified in HspB1-Null Mice. *Proteomes, 4* (2), 17.

Picard, B., Lebret, B., Cassar-Malek, I., Liaubet, L., Berri, C., Le Bihan-Duval, E., Hocquette, J. F., & Renand, G. (2015). Recent advances in omic technologies for meat quality management. *Meat Sci, 109*, 18-26.

Picard, B., Lefevre, F., & Lebret, B. (2012). Meat and fish flesh quality improvement with proteomic applications. *Animal Frontiers, 2* (4), 18-25.

Pierzchala, M., Hoekman, A. J., Urbanski, P., Kruijt, L., Kristensen, L., Young, J. F., Oksbjerg, N., Goluch, D., & te Pas, M. F. (2014). Validation of biomarkers for loin meat quality (M. longissimus) of pigs. *J Anim Breed Genet, 131* (4), 258-270.

Polati, R., Menini, M., Robotti, E., Millioni, R., Marengo, E., Novelli, E., Balzan, S., & Cecconi, D. (2012). Proteomic changes involved in tenderization of bovine Longissimus dorsi muscle during prolonged ageing. *Food Chemistry, 135* (3), 2052-2069.

Pulford, D. J., Frost, D. F., Lomiwes, D. D., & Farouk, M. M. (2008). Preliminary studies to determine the chaperoning properties of bovine casein and crystallin proteins at reducing beef muscle protein aggregation during heating. *International Journal of Food Science & Technology, 43* (12), 2143-2150.

Ramayo-Caldas, Y., Renand, G., Ballester, M., Saintilan, R., & Rocha, D. (2016). Multi-breed and multi-trait co-association analysis of meat tenderness and other meat quality traits in three French beef cattle breeds. *Genet Sel Evol, 48* (1), 37.

Rembold, C. M., Foster, D. B., Strauss, J. D., Wingard, C. J., & Eyk, J. E. (2000). cGMP-mediated phosphorylation of heat shock protein 20 may cause smooth muscle relaxation without myosin light chain dephosphorylation in swine carotid artery. *J Physiol, 524 Pt 3* (3), 865-878.

Rifai, N., Gillette, M. A., & Carr, S. A. (2006). Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol, 24* (8), 971-983.

Rodrigues, R. T. d. S., Chizzotti, M. L., Vital, C. E., Baracat-Pereira, M. C., Barros, E., Busato, K. C., Gomes, R. A., Ladeira, M. M., & Martins, T. d. S. (2017). Differences in Beef Quality between Angus (Bos taurus taurus) and Nellore (Bos taurus indicus) Cattle through a Proteomic and Phosphoproteomic Approach. *PLOS ONE, 12* (1), e0170294.

Rosa, A. F., Moncau, C., Mattos, E., Poleti, M., Balieiro, J., & Eler, J. (2017). Influence of Heat Shock Protein Activity on the Beef Tenderness during Aging. *Meat and Muscle Biology, 1* (3), 145-145.

Rosa, A. F., Moncau, C. T., Poleti, M. D., Fonseca, L. D., Balieiro, J. C. C., Silva, S. L. E., & Eler, J. P. (2018). Proteome changes of beef in Nellore cattle with different genotypes for tenderness. *Meat Sci, 138*, 1-9.

Sauerwein, H., & Meyer, H. H. D. (1989). Androgen and Estrogen Receptors in Bovine Skeletal Muscle: Relation to Steroid-Induced Allometric Muscle Growth. *Journal of Animal Science, 67* (1), 206-212.

Sedoris, K. C., Thomas, S. D., & Miller, D. M. (2010). Hypoxia induces differential translation of enolase/MBP-1. *BMC Cancer, 10*, 157.

Sierra, V., Fernandez-Suarez, V., Castro, P., Osoro, K., Vega-Naredo, I., Garcia-Macia, M., Rodriguez-Colunga, P., Coto-Montes, A., & Olivan, M. (2012). Identification of biomarkers of meat tenderisation and its use for early classification of Asturian beef into fast and late tenderising meat. *J Sci Food Agric, 92* (13), 2727-2740.

Silva, L. H. P., Rodrigues, R. T. S., Assis, D. E. F., Benedeti, P. D. B., Duarte, M. S., & Chizzotti, M. L. (2019). Explaining meat quality of bulls and steers by differential proteome and phosphoproteome analysis of skeletal muscle. *J Proteomics, 199*, 51-66.

Sorbolini, S., Marras, G., Gaspa, G., Dimauro, C., Cellesi, M., Valentini, A., & Macciotta, N. P. P. (2015). Detection of selection signatures in Piemontese and Marchigiana cattle, two breeds with similar production aptitudes but different selection histories. *Genetics Selection Evolution, 47* (1), 52.

Sørheim, O., & Hildrum, K. I. (2002). Muscle stretching techniques for improving meat tenderness. *Trends in Food Science & Technology, 13* (4), 127-135.

Suwandy, V., Carne, A., van de Ven, R., Bekhit, A. E.-D. A., & Hopkins, D. L. (2015). Effect of pulsed electric field treatment on hot-boned muscles of different potential tenderness. *Meat Sci, 105*, 25-31.

Te Pas, M. F., Hoekman, A. J., & Smits, M. A. (2011). Biomarkers as management tools for industries in the pork production chain. *Journal on Chain and Network Science, 11* (2), 155-166.

Te Pas, M. F., Kruijt, L., Pierzchala, M., Crump, R. E., Boeren, S., Keuning, E., Hoving-Bolink, R., Hortos, M., Gispert, M., Arnau, J., Diestre, A., & Mulder, H. A. (2013). Identification of proteomic biomarkers in M. Longissimus dorsi as potential predictors of pork quality. *Meat Sci, 95* (3), 679-687.

Troy, D. J., & Kerry, J. P. (2010). Consumer perception and the role of science in the meat industry. *Meat Sci, 86* (1), 214-226.

Wang, L. L., Yu, Q. L., Han, L., Ma, X. L., Song, R. D., Zhao, S. N., & Zhang, W. H. (2018). Study on the effect of reactive oxygen species-mediated oxidative stress on the activation of mitochondrial apoptosis and the tenderness of yak meat. *Food Chem, 244*, 394-402.

Wang, Y., Xu, A., & Cooper, G. J. S. (1999). Phosphorylation of P20 is associated with the actions of insulin in rat skeletal and smooth muscle. *Biochemical Journal, 344* (3), 971-976.

Warner, R. D., McDonnell, C. K., Bekhit, A. E. D., Claus, J., Vaskoska, R., Sikes, A., Dunshea, F. R., & Ha, M. (2017). Systematic review of emerging and innovative technologies for meat tenderisation. *Meat Sci, 132*, 72-89.

Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (1996). Sampling, cooking, and coring effects on Warner-Bratzler shear force values in beef2. *Journal of Animal Science, 74* (7), 1553-1562.

Woelders, H., Te Pas, M. F., Bannink, A., Veerkamp, R. F., & Smits, M. A. (2011). Systems biology in animal sciences. *Animal, 5* (7), 1036-1047.

Wu, W., Dai, R. T., & Bendixen, E. (2019). Comparing SRM and SWATH Methods for Quantitation of Bovine Muscle Proteomes. *J Agric Food Chem, 67* (5), 1608-1618.

Wu, W., Yu, Q. Q., Fu, Y., Tian, X. J., Jia, F., Li, X. M., & Dai, R. T. (2016). Towards muscle-specific meat color stability of Chinese Luxi yellow cattle: A proteomic insight into post-mortem storage. *J Proteomics, 147*, 108-118.

Wulff, T., Jokumsen, A., Højrup, P., & Jessen, F. (2012). Time-dependent changes in protein expression in rainbow trout muscle following hypoxia. *Journal of Proteomics, 75* (8), 2342-2351.

Xing, T., Gao, F., Tume, R. K., Zhou, G., & Xu, X. (2019). Stress Effects on Meat Quality: A Mechanistic Perspective. *Comprehensive Reviews in Food Science and Food Safety, 18* (2), 380-401.

Xu, Y., & López, M. (2018). Central regulation of energy metabolism by estrogens. *Molecular Metabolism, 15*, 104-115.

Xu, Y. Y., Du, F., Meng, B., Xie, G. H., Cao, J., Fan, D., & Yu, H. (2015). Hepatic overexpression of methionine sulfoxide reductase A reduces atherosclerosis in apolipoprotein E-deficient mice. *J Lipid Res, 56* (10), 1891-1900.

Yang, F., Sun, X., Beech, W., Teter, B., Wu, S., Sigel, J., Vinters, H. V., Frautschy, S. A., & Cole, G. M. (1998). Antibody to caspase-cleaved actin detects apoptosis in differentiated neuroblastoma and plaque-associated neurons and microglia in Alzheimer's disease. *Am J Pathol, 152* (2), 379-389.

Zapata, I., Zerby, H. N., & Wick, M. (2009). Functional proteomic analysis predicts beef tenderness and the tenderness differential. *J Agric Food Chem, 57* (11), 4956-4963.

Zhang, C., Wang, Z., Li, Z., Shen, Q., Chen, L., Gao, L., & Zhang, D. (2016). Phosphoproteomic profiling of myofibrillar and sarcoplasmic proteins of muscle in response to salting. *Food Science and Biotechnology, 25* (4), 993-1001.

Zhao, C., Zan, L., Wang, Y., Scott Updike, M., Liu, G., Bequette, B. J., Baldwin Vi, R. L., & Song, J. (2014). Functional proteomic and interactome analysis of proteins associated with beef tenderness in Angus cattle. *Livestock Science, 161* (0), 201-209.

Figure captions

Figure 1. Pipeline of beef tenderness biomarkers discovery followed in this meta-proteomics study. The pipeline is based on 6 phases from identification/discovery of biomarkers to the commercialization of a tool based on the list of validated biomarkers. The main proteomic workflow strategy includes 6 steps following comparative proteomics based **(1)** on extreme tenderness groups (Tender *vs.* Tough) selected using instrumental Warner-Bratzler shear force (WBSF) or sensory analysis by trained panellists on *Longissimus thoracis* (LT) or *Semitendinosus* (ST) muscles of different types of cattle: young bulls, steers and cows of several breeds. Then, **(2)** muscle protein extracts are prepared using frozen samples homogenised and centrifuged using an accurate buffer. The protein extract samples are then **(3)** subjected to 2-DE analysis where the proteins are first fractionated by isoelectrofocalisation (IEF) on 18 cm length IPG strips pH range 4 – 7 followed by a separation of the proteins in the second dimension of the SDS-PAGE gel using their molecular weights. Afterwards, **(4)** statistical comparisons are performed on the whole protein spots between tender *vs.* tough groups for each gel. Significance Analysis of Microarray (SAM) method based on a modified t-test is used to consider not only the individual variability but also the average variability of proteins with the same level of expression than the considered protein. Only the proteins with a fold change >1.5 were retained. Therefore, **(5)** the significantly differential spots were carefully excised by hand from at least three different replicate gels and placed in clean Safe-Lock Tube for distaining and preparation for identification by mass spectrometry. Finally, **(6)** protein spots are trypsin digested and subjected to identification using MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-time-of-flight) and each Peptide mass fingerprints (PMF) of trypsin-digested spots are compared to *Bos taurus* Swiss Prot database or to other mammalian using MASCOT software.

Figure 2. Enriched Gene Ontology (GO) terms of the annotated **A)** 50 putative protein biomarkers of *Longissimus thoracis* and **B)** 28 proteins of *Semitendinosus* identified in the 12 experiments. The results were generated with ProteINSIDE tool, and the protein gene names annotated by a GO term are listed.

Figure 3. Pie charts highlighting the distribution of the protein biomarkers by biological pathway for **A)** both muscles (n = 61 proteins), **B)** *Longissimus thoracis* (n = 50) and **C)** *Semitendinosus* (n = 28).

Figure 4. STRING protein-protein interaction networks (http://string-db.org) linking the annotated **A)** 50 putative protein biomarkers of *Longissimus thoracis* and **B)** 28 proteins of

Semitendinosus identified in the 12 experiments. The ProteQTL tool (http://www.proteinside.org/) revealed 14 QTL in comparison to the large literature that are shown in the interactomes by blue ovals for both muscles.

Figure 5. Pairwise protein distance (*D*) matrix expressed as a percentage based on the 61 putative protein biomarkers identified in each of the 12 proteomic experiments. The meat samples measured for tenderness by WBSF are highlighted by a grey colour and those evaluated by a sensory panel are let blank.

Figure 6. Venn diagram highlighting the distribution of 28 putative protein biomarkers from *Longissimus thoracis* muscle among Charolais young bulls (n = 22 proteins), steers (n = 9) and cows (n = 10) and related to tenderness evaluated by Warner-Bratzler Shear Force (WBSF). Two biomarkers, HSPA1B and TPI1, were common to the three Charolais animal types. The UniprotID of the proteins and direction (positive (green), negative (red), or positive/negative (orange)) of the relationships with tenderness are given in the table by biological family for all the proteins.

Figure 7. Venn diagram highlighting the distribution of 26 putative protein biomarkers from *Longissimus thoracis* muscle and young bulls related to tenderness evaluated by sensory panel from three breeds: Charolais (n = 8 proteins), Limousin (n = 14) and Blond d'Aquitaine (n = 15). Two biomarkers, ACTA1 and HSPB1, were common to the three breeds. The UniprotID of the proteins and direction (positive (green), negative (red), or positive/negative (orange)) of the relationships with tenderness are given in the table by biological family for all the proteins.

Figure 8. Venn diagram highlighting the distribution of 17 putative protein biomarkers from *Semitendinosus* muscle and young bulls related to tenderness evaluated by sensory panel from four breeds: Charolais (n = 9 proteins), Limousin (n = 4), Blond d'Aquitaine (n = 9) and Salers (n = 3). The UniprotID of the proteins and direction (positive (green) or negative (red)) of the relationships with tenderness are given in the table by biological family for all the proteins.

Figure 9. Venn diagram highlighting the distribution of 21 putative protein biomarkers from *Longissimus thoracis* muscle among Charolais (n = 10 proteins) and PDO Maine-Anjou cows (n = 17), and related to tenderness evaluated by Warner-Bratzler Shear Force (WBSF). Six biomarkers, ENO1, ENO3, MYH1, HSPB1, HSPA1B and MSRA, were common to the two cow breeds. The UniprotID of the proteins and direction (positive (green) or negative (red)) of the relationships with tenderness are given in the table by biological family for all the proteins.

Figure 10. Venn diagram highlighting the distribution of 34 putative protein biomarkers among *Longissimus thoracis* (n = 22 proteins) and *Semitendinosus* muscles (n = 16) from young Charolais bulls and related to tenderness evaluated by Warner-Bratzler Shear Force (WBSF). Four biomarkers, TPI1, HSPB6, HSPB1 and YWHAE, were common to the two muscles. The UniprotID of the proteins and direction (positive (green), negative (red), or positive/negative (orange)) of the relationships with tenderness are given in the table by biological family for all the proteins.

Figure 11. Venn diagram highlighting the distribution of 25 putative protein biomarkers from *Longissimus thoracis* muscle from young Charolais bulls comparing the proteins related to WBSF (n = 22 proteins) and sensory panel (n = 8). Five biomarkers, PGM1, ACTA1, TnnT3, HSPB1 and PARK7, were common to the two evaluation methods of tenderness. The UniprotID of the proteins and direction (positive (green), negative (red), or positive/negative (orange)) of the relationships with tenderness are given in the table by biological family for all the proteins.

Figure 12. Venn diagram highlighting the distribution of 22 putative protein biomarkers from *Semitendinosus* muscle from young Charolais bulls comparing the proteins related to WBSF (n = 16 proteins) and sensory panel (n = 9). Three biomarkers, MYL2, HSPB1 and PVALB, were common to the two evaluation methods of tenderness. The UniprotID of the proteins and direction (positive (green) or negative (red)) of the relationships with tenderness are given in the table by biological family for all the proteins.

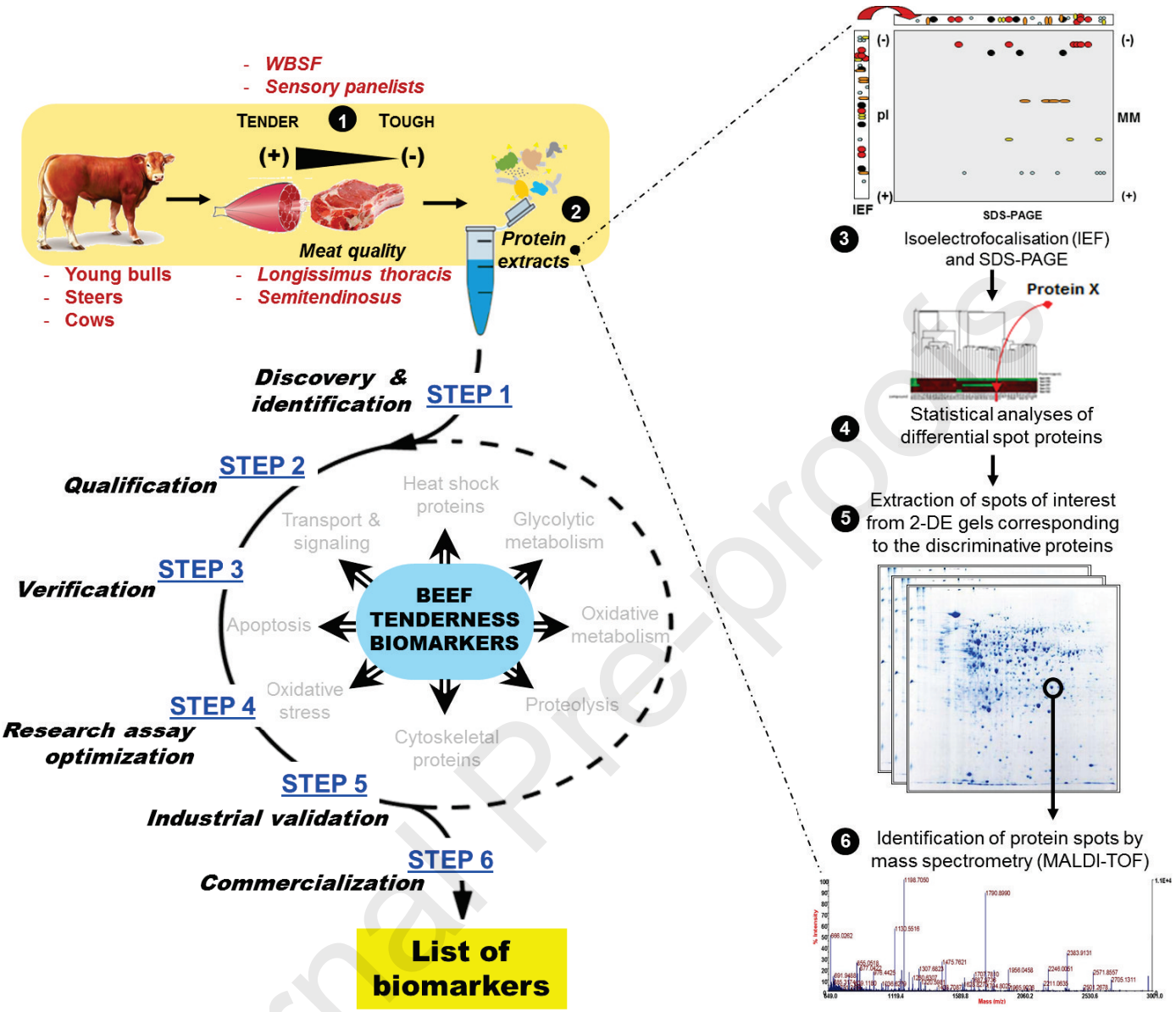
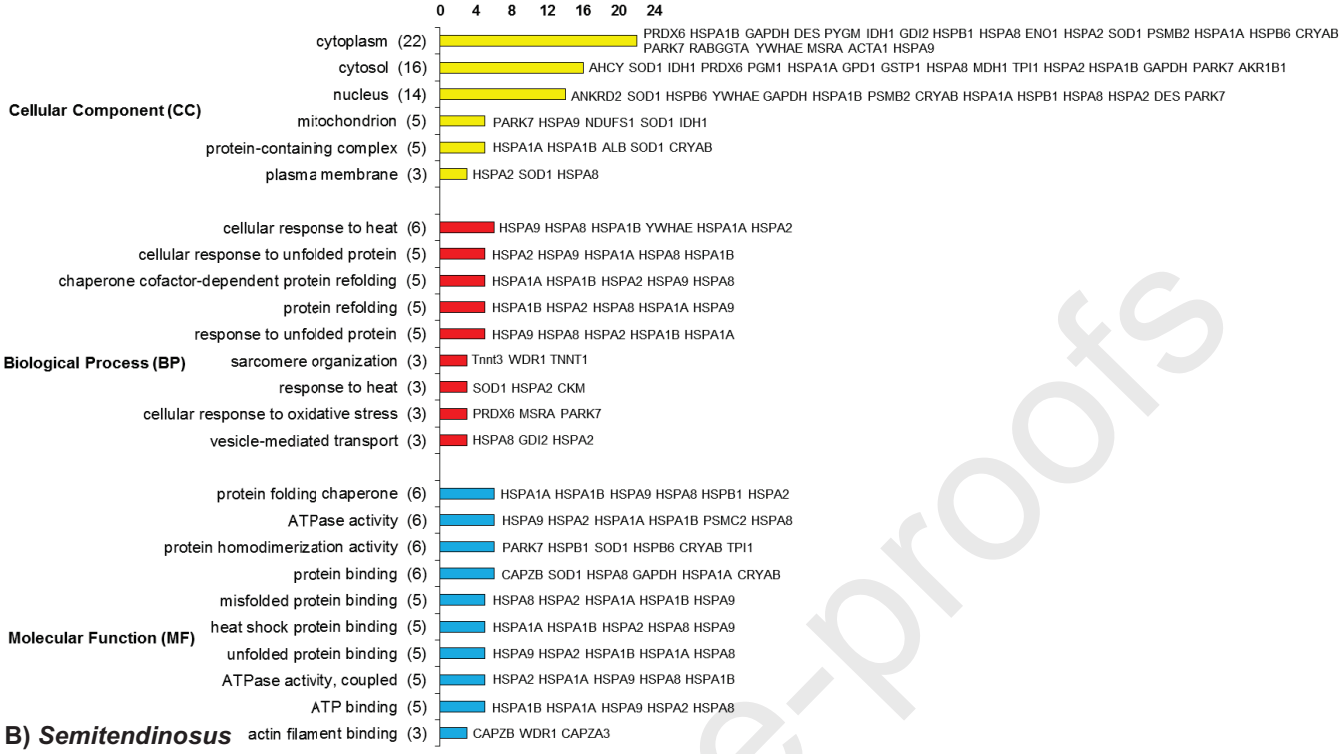

- WBSF
- Sensory panelists
TENDER 1 TOUGH
(+) (-)
Meat quality
- Young bulls
- Steers
- Cows
- Longissimus thoracis
- Semitendinosus
2
Protein extracts
Discovery & identification
STEP 1
STEP 2
Qualification
STEP 3
Verification
STEP 4
Research assay optimization
STEP 5
Industrial validation
STEP 6
Commercialization
BEEF TENDERNESS BIOMARKERS
Heat shock proteins
Glycolytic metabolism
Oxidative metabolism
Proteolysis
Cytoskeletal proteins
Oxidative stress
Apoptosis
Transport & signaling
List of biomarkers
IEF
SDS-PAGE
pI
MM
3 Isoelectrofocalisation (IEF) and SDS-PAGE
Protein X
4 Statistical analyses of differential spot proteins
5 Extraction of spots of interest from 2-DE gels corresponding to the discriminative proteins
6 Identification of protein spots by mass spectrometry (MALDI-TOF)

Figure 1.

A) *Longissimus thoracis*



B) *Semitendinosus*

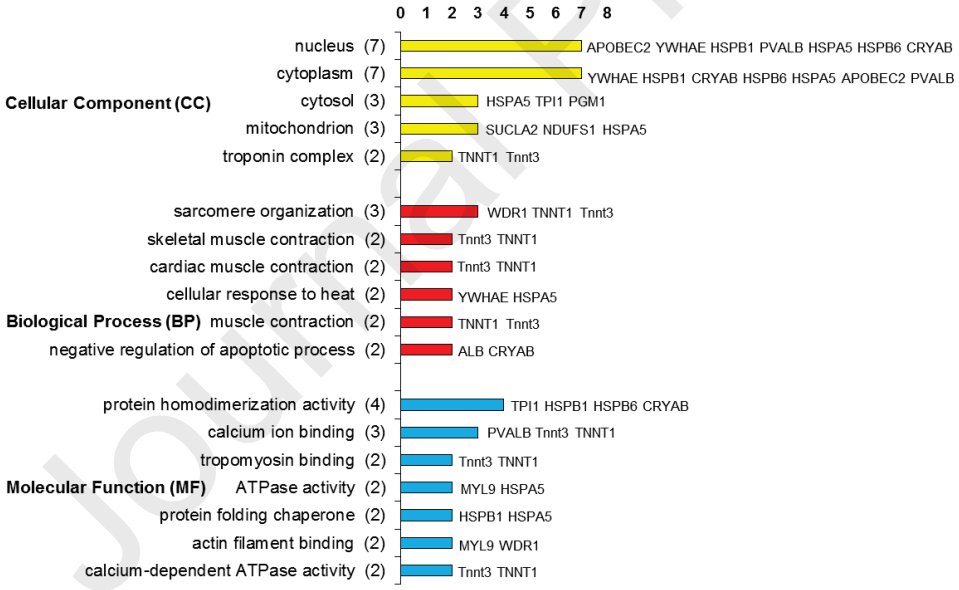


Figure 2.

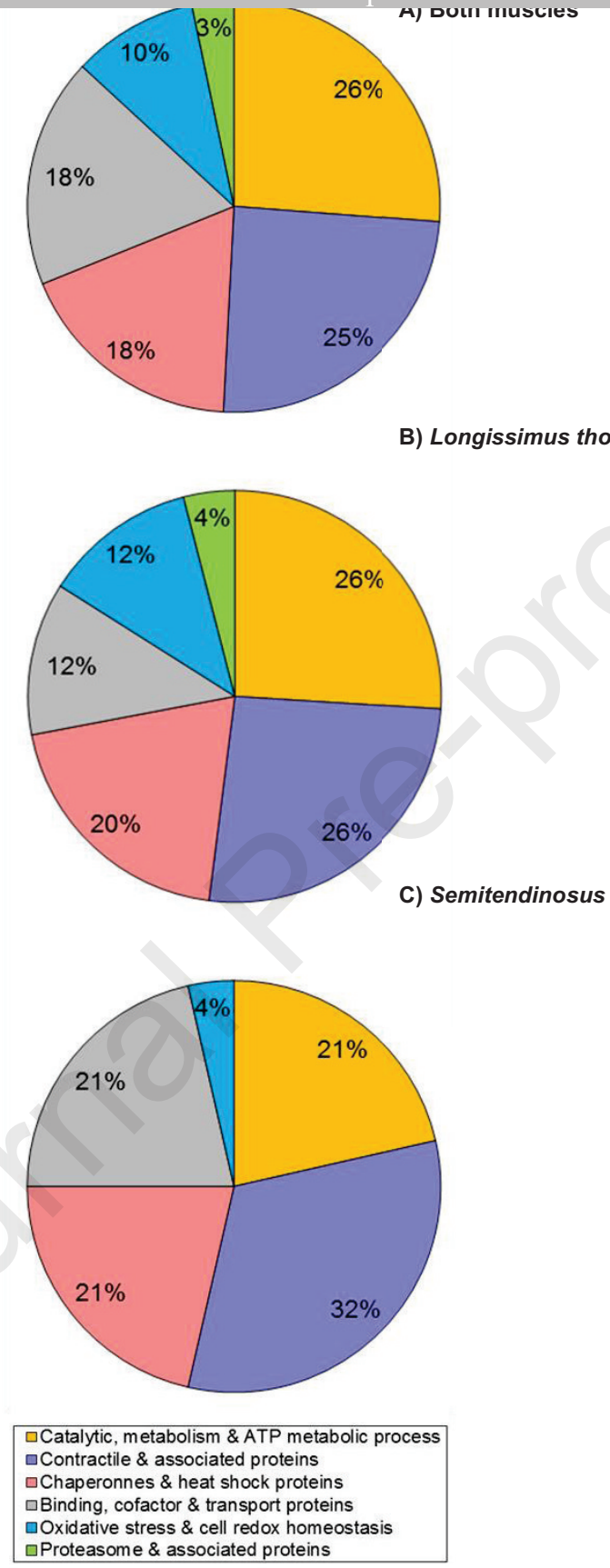


Figure 3.

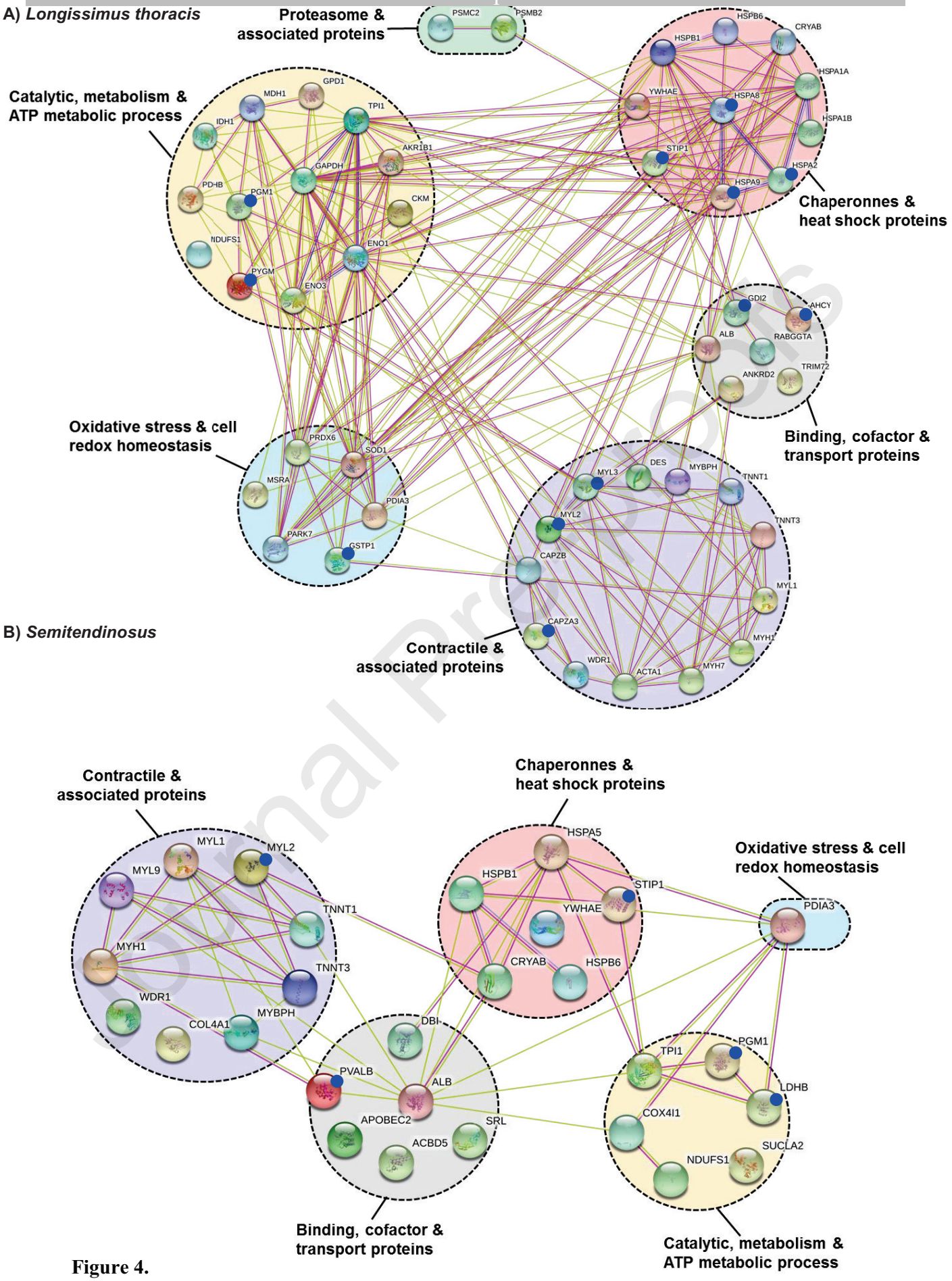

A) Longissimus thoracis
Proteasome & associated proteins
PSMC2
PSMB2
Catalytic, metabolism & ATP metabolic process
GPD1
MDH1
TPI1
IDH1
AKR1B1
GAPDH
PDHB
PGM1
CKM
NDUFS1
ENO1
PYGM
ENO3
HSPB6
CRYAB
HSPB1
HSPA1A
YWHAE
HSPA8
HSPA1B
STIP1
HSPA2
HSPA9
Chaperonnes & heat shock proteins
GDI2
AHCY
ALB
RABGGTA
ANKRD2
TRIM72
Binding, cofactor & transport proteins
Oxidative stress & cell redox homeostasis
PRDX6
SOD1
MSRA
PDIA3
PARK7
GSTP1
MYL3
DES
MYBPH
TNNT1
MYL2
TNNT3
CAPZB
MYL1
CAPZA3
MYH1
WDR1
ACTA1
MYH7
Contractile & associated proteins
B) Semitendinosus
Contractile & associated proteins
MYL1
MYL2
MYL9
TNNT1
MYH1
TNNT3
WDR1
MYBPH
COL4A1
Chaperonnes & heat shock proteins
HSPA5
HSPB1
STIP1
YWHAE
CRYAB
HSPB6
Oxidative stress & cell redox homeostasis
PDIA3
DBI
PVALB
ALB
APOBEC2
SRL
ACBD5
Binding, cofactor & transport proteins
TPI1
PGM1
LDHB
COX4I1
SUCLA2
NDUFS1
Catalytic, metabolism & ATP metabolic process

Figure 4.

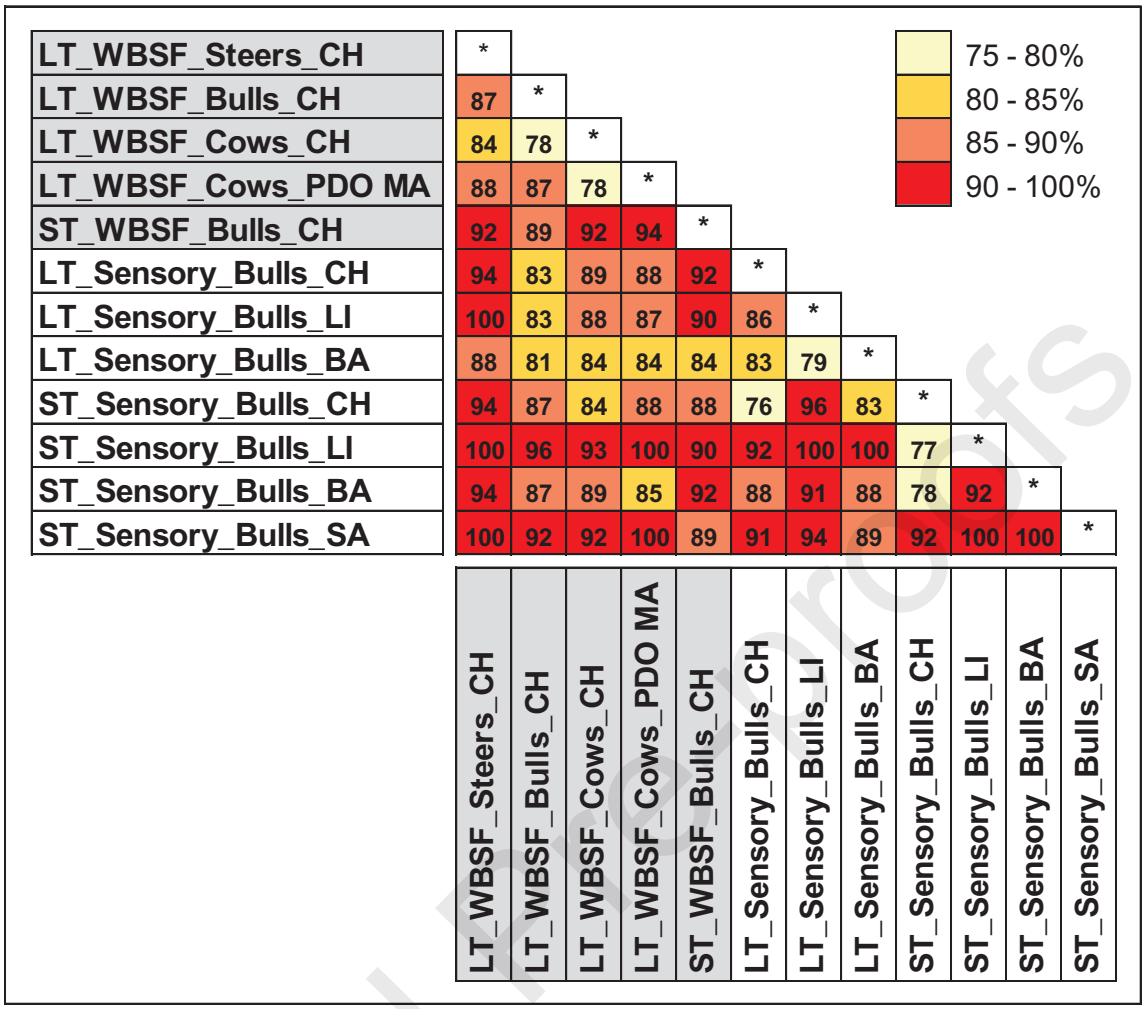
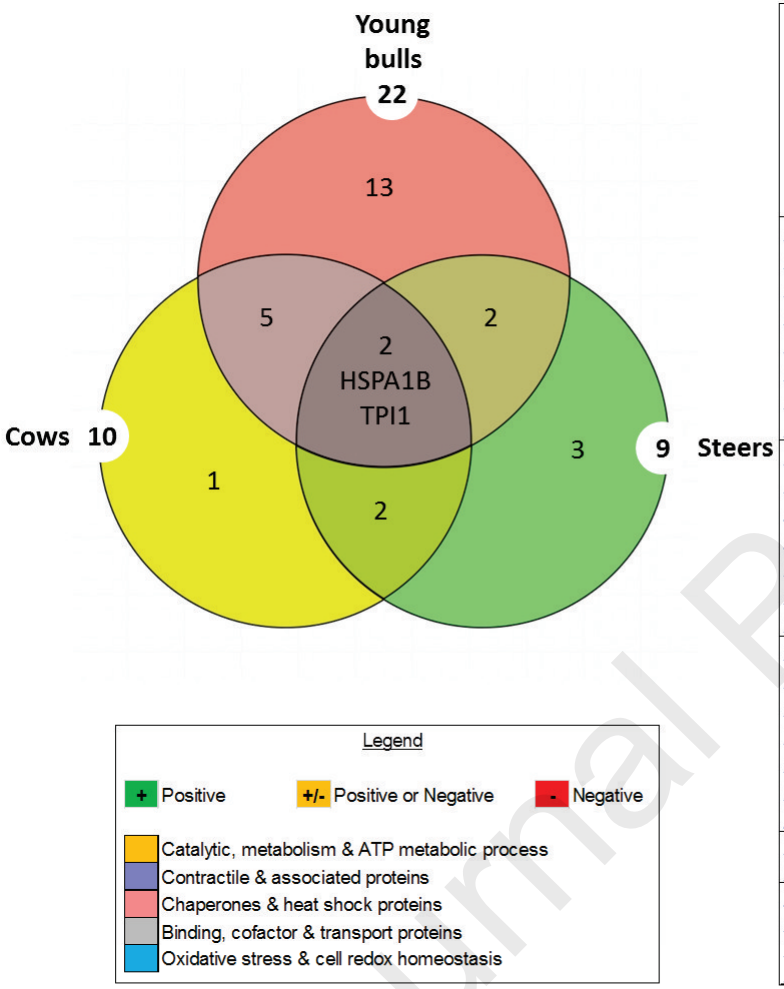
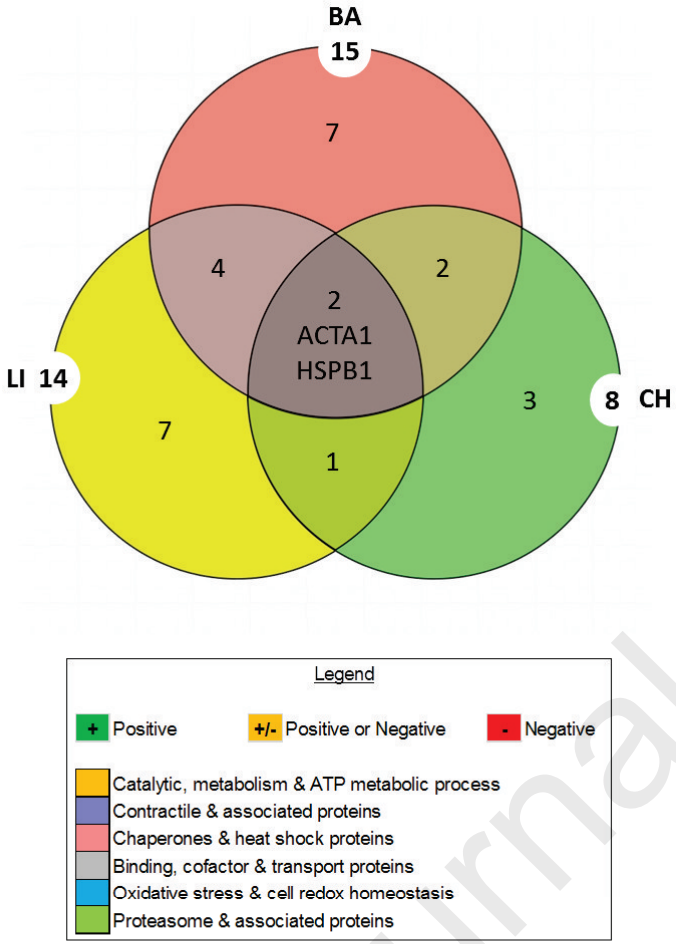


Figure 5.



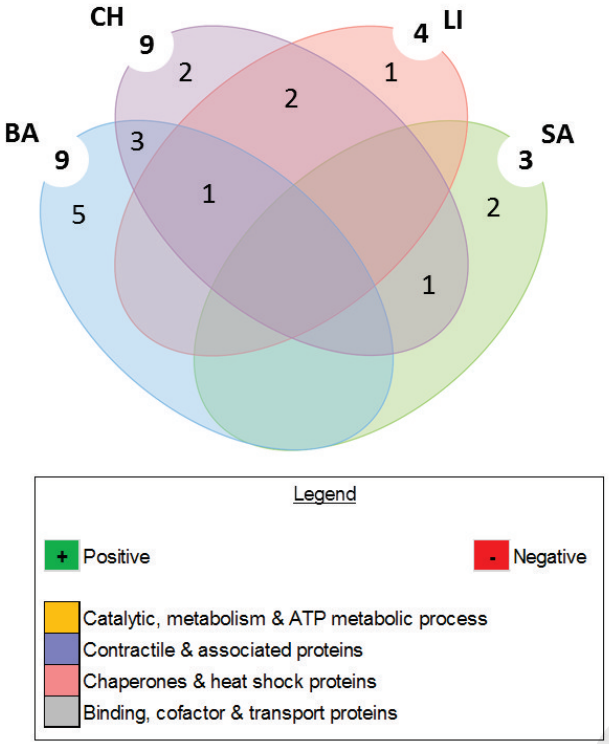
Uniprot ID	Protein name	Gene name	LT_WBSF_Steers_CH	LT_WBSF_Bulls_CH	LT_WBSF_Cows_CH
Q9XSJ4	Alpha-enolase	ENO1		-	+
Q3ZC09	Beta-enolase	ENO3			+
Q3T145	Malate dehydrogenase, cytoplasmic	MDH1		-	
Q08DP0	Phosphoglucomutase-1	PGM1		-	+
P79334	Glycogen phosphorylase	PYGM		-	
Q5E956	Triosephosphate isomerase	TPI1	+	-	+
Q5EA88	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	GPD1	+		
P16116	Aldo-keto reductase family 1 member B1	AKR1B1	-		
P68138	Actin, alpha skeletal muscle	ACTA1		+/-	
P79136	F-actin-capping protein subunit beta	CAPZB		-	
Q9BE39	Myosin-7	MyH7		+	
Q9BE40	Myosin-1	MyH1		+	-
Q0VBZ1	Myosin binding protein H	MYBPH		-	-
Q8MKI3	Troponin T, Fast	TnnT3	+	-	
O62654	Desmin	DES		-	
Q3ZCH0	Stress-70 protein, mitochondrial (GRP75)	HSPA9	-		-
Q148F8	Heat shock protein beta-6 (Hsp20)	HSPB6		+	
Q3T149	Heat shock protein beta-1 (Hsp27)	HSPB1		+/-	+
P34933	Heat shock-related 70 kDa protein 2	HSPA2		-	
Q27975	Heat shock 70 kDa protein 1A	HSPA1A	+		
Q27965	Heat shock 70 kDa protein 1B	HSPA1B	-	-	-
P62261	14-3-3 protein epsilon	YWHAE	+	-	
P02769	Serum albumin	ALB		-	
Q3MHL4	Adenosylhomocysteinase	AHCY		-	
P00442	Superoxide dismutase [Cu-Zn]	SOD1		-	
O77834	Peroxiredoxin 6	PRDX6		-	
Q5E946	Protein/nucleic acid deglycase DJ-1	PARK7		-	
P54149	Mitochondrial peptide methionine sulfoxide reductase	MSRA	+		+
		Total	9	22	10

Figure 6.



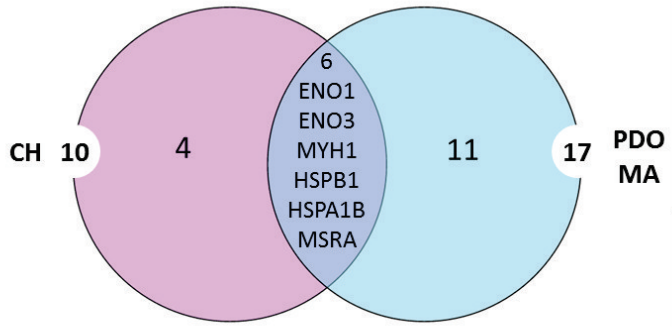
Uniprot ID	Protein name	Gene name	LT_Sensory_Bulls_CH	LT_Sensory_Bulls_LI	LT_Sensory_Bulls_BA
Q9XSC6	Creatine kinase M-type	CKM			+
Q9XSJ4	Alpha-enolase	ENO1		+	
Q3ZC09	Beta-enolase	ENO3		-	-
P10096	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH		-	
Q08DP0	Phosphoglucomutase-1	PGM1	-		-
P15690	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	NDUFS1			+
P68138	Actin, alpha skeletal muscle	ACTA1	+	+	+/-
Q32KS3	F-actin-capping protein subunit alpha	CAPZA3		+	
P79136	F-actin-capping protein subunit beta	CAPZB		-	+
Q3SZE5	Myosin regulatory light chain 2, skeletal muscle isoform type 1	MYL2	-		
P85100	Myosin light chain3,	MYL3			+
Q8MKI3	Troponin T, Fast	TnnT3	-		-
Q8MKH6	Troponin T, slow	TnnT1			-
Q2KJH4	WD repeat-containing protein 1	WDR1			-
Q3ZCH0	Stress-70 protein, mitochondrial (GRP75)	HSPA9			+
Q148F8	Heat shock protein beta-6 (Hsp20)	HSPB6		+/-	-
Q3T149	Heat shock protein beta-1 (Hsp27)	HSPB1	+/-	+/-	+/-
P31948	Stress-induced-phosphoprotein 1	STIP1		-	
P02510	Alpha-crystallin B chain	CRYAB		+	-
P62261	14-3-3 protein epsilon	YWHAE			+
P50397	Rab GDP dissociation inhibitor beta	GDI2		+	
Q5EA80	Geranylgeranyl transferase type-2 subunit alpha	RABGGTA		-	
P28801	Glutathione S-transferase P	GSTP1	+		
Q5E946	Protein/nucleic acid deglycase DJ-1	PARK7	+	-	
Q5E9K0	Proteasome subunit beta type-2	PSMB2	+		
Q5E9F9	26S proteasome regulatory subunit 7	PSMC2		+	
		Total	8	14	15

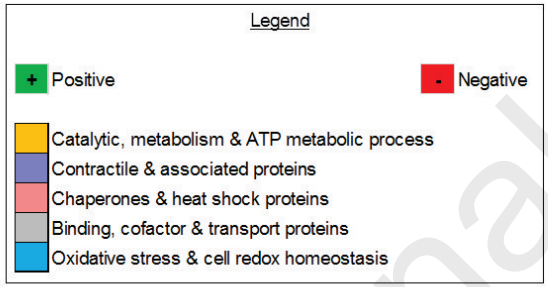
Figure 7.



Uniprot ID	Protein name	Gene name	ST_Sensory_Bulls_BA	ST_Sensory_Bulls_CH	ST_Sensory_Bulls_LI	ST_Sensory_Bulls_SA
Q5E9B1	L-lactate dehydrogenase B chain	LDHB				-
Q08DP0	Phosphoglucomutase-1	PGM1		-		-
P00423	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	COX4I1	+			
Q9BE40	Myosin-1	MyH1	+			
Q3SZE5	Myosin regulatory light chain 2, skeletal muscle isoform type 1	MYL2		-	-	
Q0VBZ1	Myosin binding protein H	MYBPH		+	+	
A0JNJ5	Myosin light chain 1A,slow isoforme	MYL1	+	+		
Q5E9E2	Myosin regulatory light chain2,phosphorylée MLC2-P	MYRL2		+		
Q8MKI3	Troponin T, Fast	TnnT3	+	+		
Q8MKH6	Troponin T, slow	TnnT1		+		
Q7SIB2	collagen alpha-1(iv) chain	COL4A1	+			
Q148F8	Heat shock protein beta-6 (Hsp20)	HSPB6				-
Q3T149	Heat shock protein beta-1 (Hsp27)	HSPB1	+	-		
P02510	Alpha-crystallin B chain	CRYAB	+			
P07106	Acyl-CoA-binding domain-containing protein 5	ACBD5			+	
P02769	Serum albumin	ALB	+			
Q0VCG3	Parvalbumin alpha	PVALB	-	+	+	
		Total	9	9	4	3

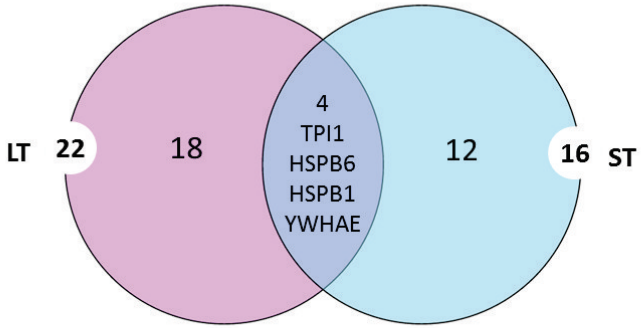
Figure 8.

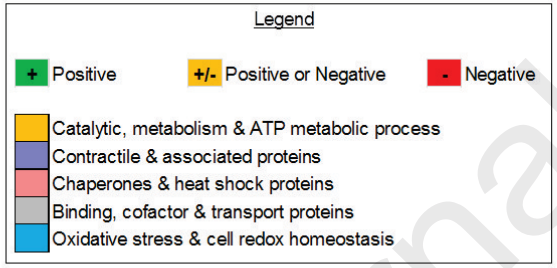




Uniprot ID	Protein name	Gene name	LT_WBSF_Cows_CH	LT_WBSF_Cows_PDO MA
Q9XSJ4	Alpha-enolase	ENO1	+	+
Q3ZC09	Beta-enolase	ENO3	+	+
Q9XSG3	Isocitrate dehydrogenase [NADP] cytoplasmic	IDH1		+
P11966	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	PDHB		+
Q08DP0	Phosphoglucomutase-1	PGM1	+	
Q5E956	Triosephosphate isomerase	TPI1	+	
P68138	Actin, alpha skeletal muscle	ACTA1		+
Q9BE40	Myosin-1	MyH1	-	-
Q0VBZ1	Myosin binding protein H	MYBPH	-	
A0JNJ5	Myosin light chain 1A,slow isoforme	MYL1		+
P85100	Myosin light chain3,	MYL3		+
Q8MKI3	Troponin T, Fast	TnnT3		+
Q3ZCH0	Stress-70 protein, mitochondrial (GRP75)	HSPA9	-	
Q3T149	Heat shock protein beta-1 (Hsp27)	HSPB1	+	-
P19120	Heat shock cognate 71 kDa protein	HSPA8		+
Q27975	Heat shock 70 kDa protein 1A	HSPA1A		+
Q27965	Heat shock 70 kDa protein 1B	HSPA1B	-	-
F1MX12	Ankyrin repeat domain 2	ANKRD2		+
E1BE77	Tripartite motif-containing protein 72	TRIM72		-
P54149	Mitochondrial peptide methionine sulfoxide reductase	MSRA	+	+
P38657	Protein disulfide-isomerase A3	PDIA3		-
		Total	10	17

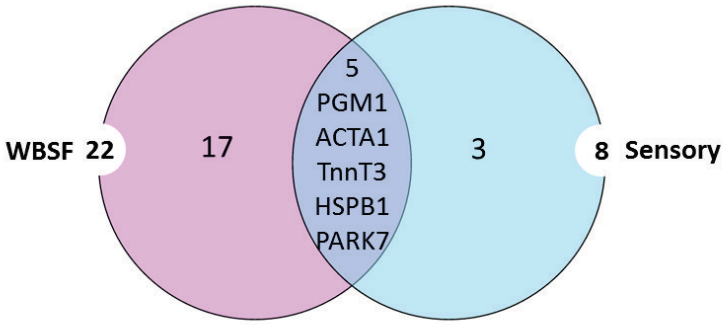
Figure 9.

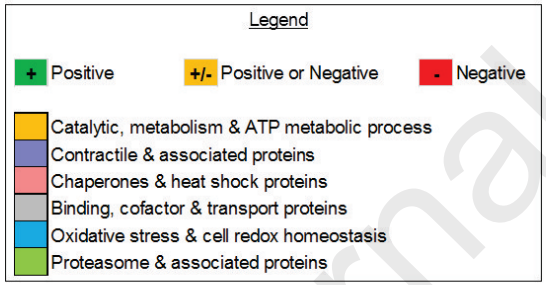




Uniprot ID	Protein name	Gene name	LT_WBSF_Bulls_CH	ST_WBSF_Bulls_CH
Q9XSJ4	Alpha-enolase	ENO1	-	
Q3T145	Malate dehydrogenase, cytoplasmic	MDH1	-	
Q5E9B1	L-lactate dehydrogenase B chain	LDHB		+
Q08DP0	Phosphoglucomutase-1	PGM1	-	
P79334	Glycogen phosphorylase	PYGM	-	
Q5E956	Triosephosphate isomerase	TPI1	-	+
F1MGC0	Succinate--CoA ligase [ADP-forming] subunit beta, mitochondrial	SUCLA2		+
P15690	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	NDUFS1		+
P68138	Actin, alpha skeletal muscle	ACTA1	+/-	
P79136	F-actin-capping protein subunit beta	CAPZB	-	
Q9BE39	Myosin-7	MyH7	+	
Q9BE40	Myosin-1	MyH1	+	
Q3SZE5	Myosin regulatory light chain 2, skeletal muscle isoform type 1	MYL2		-
Q0VBZ1	Myosin binding protein H	MYBPH	-	
Q8MKI3	Troponin T, Fast	TnnT3	-	
O62654	Desmin	DES	-	
Q2KJH4	WD repeat-containing protein 1	WDR1		+
Q0VCX2	Endoplasmic reticulum chaperone BiP (GRP78)	HSPA5		+
Q148F8	Heat shock protein beta-6 (Hsp20)	HSPB6	+	+
Q3T149	Heat shock protein beta-1 (Hsp27)	HSPB1	+/-	-
P34933	Heat shock-related 70 kDa protein 2	HSPA2	-	
Q27965	Heat shock 70 kDa protein 1B	HSPA1B	-	
P31948	Stress-induced-phosphoprotein 1	STIP1		+
P62261	14-3-3 protein epsilon	YWHAE	-	+
Q3SYR3	Probable C->U-editing enzyme APOBEC-2	APOBEC2		+
P07107	Acyl-CoA-binding protein	DBI		+
P02769	Serum albumin	ALB	-	
Q3MHL4	Adenosylhomocysteinase	AHCY	-	
Q86TD4	Sarcalumenin	SRL		+
Q0VCG3	Parvalbumin alpha	PVALB		+
P00442	Superoxide dismutase [Cu-Zn]	SOD1	-	
O77834	Peroxiredoxin 6	PRDX6	-	
Q5E946	Protein/nucleic acid deglycase DJ-1	PARK7	-	
P38657	Protein disulfide-isomerase A3	PDIA3		+
		Total	22	16

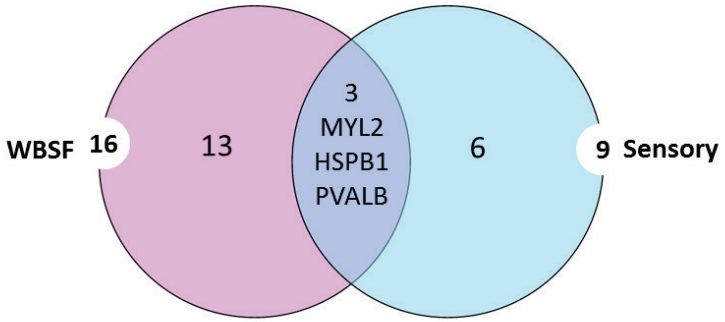
Figure 10.

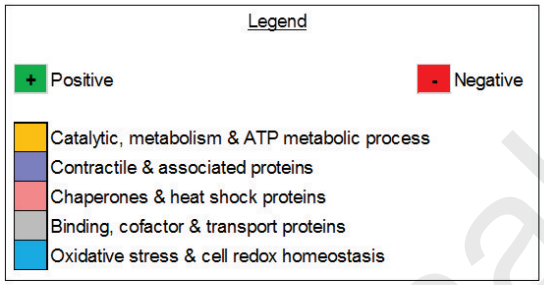




Uniprot ID	Protein name	Gene name	LT_WBSF_Bulls_CH	LT_Sensory_Bulls_CH
Q9XSJ4	Alpha-enolase	ENO1	-	
Q3T145	Malate dehydrogenase, cytoplasmic	MDH1	-	
Q08DP0	Phosphoglucomutase-1	PGM1	-	-
P79334	Glycogen phosphorylase	PYGM	-	
Q5E956	Triosephosphate isomerase	TPI1	-	
P68138	Actin, alpha skeletal muscle	ACTA1		+
P79136	F-actin-capping protein subunit beta	CAPZB	-	
Q9BE39	Myosin-7	MyH7	+	
Q9BE40	Myosin-1	MyH1	+	
Q3SZE5	Myosin regulatory light chain 2, skeletal muscle isoform type 1	MYL2		-
Q0VBZ1	Myosin binding protein H	MYBPH	-	
Q8MKI3	Troponin T, Fast	TnnT3	-	-
O62654	Desmin	DES	-	
Q148F8	Heat shock protein beta-6 (Hsp20)	HSPB6	+	
Q3T149	Heat shock protein beta-1 (Hsp27)	HSPB1		
P34933	Heat shock-related 70 kDa protein 2	HSPA2	-	
Q27965	Heat shock 70 kDa protein 1B	HSPA1B	-	
P62261	14-3-3 protein epsilon	YWHAE	-	
P02769	Serum albumin	ALB	-	
Q3MHL4	Adenosylhomocysteinase	AHCY	-	
P28801	Glutathione S-transferase P	GSTP1		+
P00442	Superoxide dismutase [Cu-Zn]	SOD1	-	
O77834	Peroxiredoxin 6	PRDX6	-	
Q5E946	Protein/nucleic acid deglycase DJ-1	PARK7	-	+
Q5E9K0	Proteasome subunit beta type-2	PSMB2		+
		Total	22	8

Figure 11.





Uniprot ID	Protein name	Gene name	ST_WBSF_Bulls_CH	ST_Sensory_Bulls_CH
Q5E9B1	L-lactate dehydrogenase B chain	LDHB	+	
Q08DP0	Phosphoglucomutase-1	PGM1		-
Q5E956	Triosephosphate isomerase	TPI1	+	
F1MGC0	Succinate--CoA ligase [ADP-forming] subunit beta, mitochondrial	SUCLA2	+	
P15690	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	NDUFS1	+	
Q3SZE5	Myosin regulatory light chain 2, skeletal muscle isoform type 1	MYL2	-	-
Q0VBZ1	Myosin binding protein H	MYBPH		+
A0JNJ5	Myosin light chain 1A,slow isoforme	MYL1		+
Q5E9E2	Myosin regulatory light chain2,phosphorylée MLC2-P	MYRL2		+
Q8MKI3	Troponin T, Fast	TnnT3		+
Q8MKH6	Troponin T, slow	TnnT1		+
Q2KJH4	WD repeat-containing protein 1	WDR1	+	
Q0VCX2	Endoplasmic reticulum chaperone BiP (GRP78)	HSPA5	+	
Q148F8	Heat shock protein beta-6 (Hsp20)	HSPB6	+	
Q3T149	Heat shock protein beta-1 (Hsp27)	HSPB1	-	-
P31948	Stress-induced-phosphoprotein 1	STIP1	+	
P62261	14-3-3 protein epsilon	YWHAE	+	
Q3SYR3	Probable C->U-editing enzyme APOBEC-2	APOBEC2	+	
P07107	Acyl-CoA-binding protein	DBI	+	
Q86TD4	Sarcalumenin	SRL	+	
Q0VCG3	Parvalbumin alpha	PVALB	+	+
P38657	Protein disulfide-isomerase A3	PDIA3	+	
		Total	16	9

Figure 12.

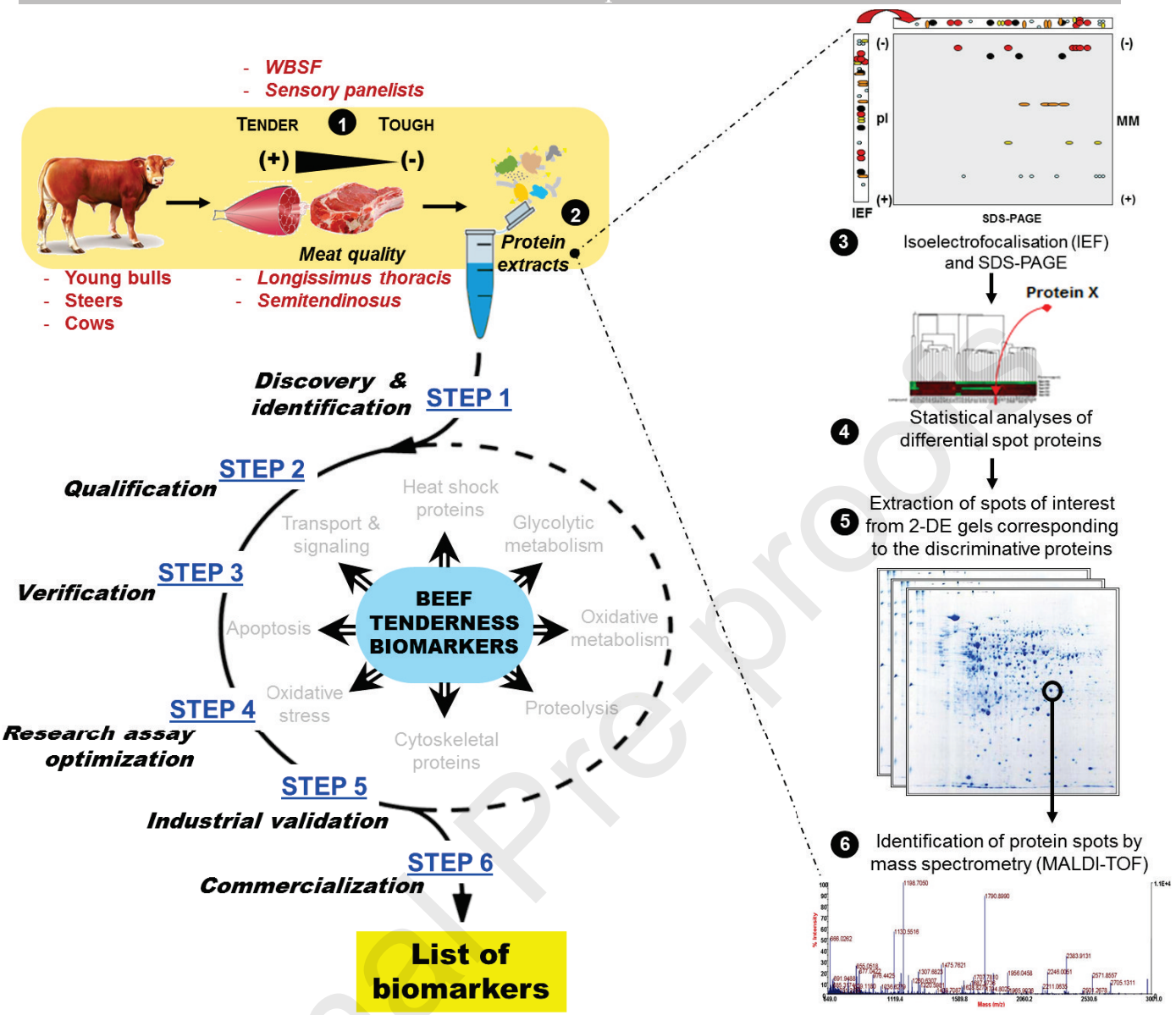
Table 1. Brief description of the experimental designs, muscle and breeds and proteomics conditions followed in the 12 proteomic studies.

	Study 1	Study 2	Study 3	Study 4	Study 5	Study 6	Study 7	Study 8	Study 9	Study 10	Study 11	Study 12
References of the experimental design	Guillemin *et al.* 2011	Guillemin *et al.* 2011	Aviles *et al.* 2014	Gagaoua *et al.* 2017 Couvreur *et al.* 2019	Guillemin *et al.* 2011	Chaze *et al.* 2013	Chaze *et al.* 2013	Chaze *et al.* 2013	Jurie *et al.* 2005	Jurie *et al.* 2005	Morzel *et al.* 2008 Picard *et al.* 2006	Jurie *et al.* 2005
Characteristics	**LT_WBSF_Steers_CH**	**LT_WBSF_Bulls_CH**	**LT_WBSF_Cows_CH**	**LT_WBSF_Cows_PDO MA**	**ST_WBSF_Bulls_CH**	**LT_Sensory_Bulls_CH**	**LT_Sensory_Bulls_LI**	**LT_Sensory_Bulls_BA**	**ST_Sensory_Bulls_CH**	**ST_Sensory_Bulls_LI**	**ST_Sensory_Bulls_BA**	**ST_Sensory_Bulls_SA**
Breed	Charolais	Charolais	Charolais	PDO Maine Anjou	Charolais	Charolais	Limousin	Blond d'Aquitaine	Charolais	Limousin	Blond d'Aquitaine	Salers
Animal type	Steers	Bulls	Cows	Cows	Bulls	Bulls	Bulls	Bulls	Bulls	Bulls	Bulls	Bulls
Age at slaughter	30 months	17 months	86 months	67 months	17 months	16 months	16 months	15 months	15 months	15 months	15 months	15 months
Muscle and sampling position	*Longissimus thoracis,* between 6^{th} & 7^{th} rib	*Longissimus thoracis,* between 6^{th} & 7^{th} rib	*Longissimus thoracis,* between 6^{th} & 7^{th} rib	*Longissimus thoracis,* between 6^{th} & 7^{th} rib	*Semitendinosus* in the center	*Longissimus thoracis,* between 6^{th} & 7^{th} rib	*Longissimus thoracis,* between 6^{th} & 7^{th} rib	*Longissimus thoracis,* between 6^{th} & 7^{th} rib	*Semitendinosus* in the center	*Semitendinosus* in the center	*Semitendinosus i* n the center	*Semitendinosus* in the center
Sampling time	15 min *post-mortem*	15 min *post-mortem*	60 min *post-mortem*	24 h *post-mortem*	15 min *post-mortem*	24 h *post-mortem*	24 h post-mortem	24 h post-mortem	15 min *post-mortem*	15 min *post-mortem*	60 min *post-mortem*	15 min *post-mortem*
Ageing	21 days	21 days	14 days	14 days	21 days	14 days	14 days	14 days	14 days	14 days	14 days	14 days
Sensory evaluation, 55°C	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sensory scores (0 - 10) of TE *vs.* TO	-	-	-	-	-	7.24 ± 0.40 *vs.* 5.0 ± 0.65	7.17 ± 0.65 *vs.* 4.4 ± 4.97	5.52 ± 0.2 *vs.* 3.73 ± 0.5	6.52 ± 0.0 *vs.* 4.36 ± 0.3	7.46 ± 0.9 *vs.* 4.26 ± 0.2	5.10 ± 0.2 *vs.* 3.92 ± 0.1	6.64 ± 0.3 *vs.* 4.21 ± 0.1
WBSF	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Shear force values, N/cm² (unless study 3 which is in kg) of TE *vs.* TO	23.4 ± 2.3 *vs.* 43.2 ± 3.5	27.7 ± 4.9 *vs.* 44.5 ± 6.1	1.75 ± 0.4 *vs.* 3.44 ± 0.2	27.9 ± 2.7 *vs.* 69.6 ± 7.6	33.7 ± 2.97 vs. 81.3 ± 8.55	30.9 ± 5.4 *vs.* 57.0 ± 16.3	32.2± 4.75 *vs.* 59.2 ± 9.8	45.6 ± 9.3 *vs.* 78.3 ± 5.5	-	-	-	-
Number of samples in TE *vs.* TO	6 (3 + 3)	8 (4 + 4)	10 (5 + 5)	10 (5 + 5)	8 (4 + 4)	20 (10 + 10)	20 (10 + 10)	20 (10 + 10)	4 (2 + 2)	4 (2 + 2)	6 (3 + 3)	4 (2 + 2)
Protein extract	Myofibrillar & sarcoplasmic proteins											
Proteomic approach	2DE + MALDI-TOF/TOF											
IPG strips	18 cm, pH 4 - 7											

Table 2. List of the 61 putative protein biomarkers by biological pathway identified in the 12 experiments.[1]

Uniprot ID	Protein name	Gene name	LT_WBSF_Steers_CH	LT_WBSF_Bulls_CH	LT_WBSF_Cows_CH	LT_WBSF_Cows_PDO MA	ST_WBSF_Bulls_CH	LT_Sensory_Bulls_CH	LT_Sensory_Bulls_LI	LT_Sensory_Bulls_BA	ST_Sensory_Bulls_CH	ST_Sensory_Bulls_LI	ST_Sensory_Bulls_BA	ST_Sensory_Bulls_SA	
Q9XSC6	Creatine kinase M-type	CKM								+					18 times +
Q9XSJ4	Alpha-enolase	ENO1		-	+	+			+						14 times -
Q3ZC09	Beta-enolase	ENO3			+	+			-	-					
P10096	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH							-						
Q9XSG3	Isocitrate dehydrogenase [NADP] cytoplasmic	IDH1				+									
Q3T145	Malate dehydrogenase, cytoplasmic	MDH1		-											
Q5E9B1	L-lactate dehydrogenase B chain	LDHB					+							-	
P11966	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	PDHB				+									
Q08DP0	Phosphoglucomutase-1	PGM1		-	+			-		-	-			-	
P79334	Glycogen phosphorylase	PYGM		-											
Q5E956	Triosephosphate isomerase	TPI1	+	-	+		+								
Q5EA88	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	GPD1	+												
P16116	Aldo-keto reductase family 1 member B1	AKR1B1	-												
F1MGC0	Succinate--CoA ligase [ADP-forming] subunit beta, mitochondrial	SUCLA2					+								
P00423	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	COX4I1											+		
P15690	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	NDUFS1					+			+					
P68138	Actin, alpha skeletal muscle	ACTA1		~		+		+	+	~					23 times +
Q32KS3	F-actin-capping protein subunit alpha	CAPZA3							+						16 times -
P79136	F-actin-capping protein subunit beta	CAPZB		-					-	+					2 times +/-
Q9BE39	Myosin-7	MyH7		+											
Q9BE40	Myosin-1	MyH1		+	-	-							+		
Q3SZE5	Myosin regulatory light chain 2, skeletal muscle isoform type 1	MYL2					-	-			-	-			
Q0VBZ1	Myosin binding protein H	MYBPH		-	-						+	+			
A0JNJ5	Myosin light chain 1A,slow isoforme	MYL1				+					+		+		
Q5E9E2	Myosin regulatory light chain2,phosphorylée MLC2-P	MYRL2									+				
P85100	Myosin light chain3,	MYL3				+				+					
Q8MKI3	Troponin T, Fast	TnnT3	+	-		+		-		-	+		+		
Q8MKH6	Troponin T, slow	TnnT1								-	+				
O62654	Desmin	DES		-											
Q2KJH4	WD repeat-containing protein 1	WDR1					+			-					
Q7SIB2	Collagen alpha-1(iv) chain	COL4A1											+		
Q3ZCH0	Stress-70 protein, mitochondrial (GRP75)	HSPA9	-		-					+					15 times +
Q0VCX2	Endoplasmic reticulum chaperone BiP (GRP78)	HSPA5					+								15 times -
Q148F8	Heat shock protein beta-6 (Hsp20)	HSPB6		+			+		~	-				-	5 times +/-
Q3T149	Heat shock protein beta-1 (Hsp27)	HSPB1		~	+	-	-	~	~	~	-		+		
P34933	Heat shock-related 70 kDa protein 2	HSPA2		-											
P19120	Heat shock cognate 71 kDa protein	HSPA8				+									
Q27975	Heat shock 70 kDa protein 1A	HSPA1A	+			+									
Q27965	Heat shock 70 kDa protein 1B	HSPA1B	-	-	-	-									
P31948	Stress-induced-phosphoprotein 1	STIP1					+		-						
P02510	Alpha-crystallin B chain	CRYAB							+	-			+		
P62261	14-3-3 protein epsilon	YWHAE	+	-			+			+					
P07106	Acyl-CoA-binding domain-containing protein 5	ACBD5										+			10 times +
F1MX12	Ankyrin repeat domain 2	ANKRD2				+									5 times -
Q3SYR3	Probable C->U-editing enzyme APOBEC-2	APOBEC2					+								
P07107	Acyl-CoA-binding protein	DBI					+								
P50397	Rab GDP dissociation inhibitor beta	GDI2							+						
P02769	Serum albumin	ALB		-									+		
Q5EA80	Geranylgeranyl transferase type-2 subunit alpha	RABGGTA							-						
E1BE77	Tripartite motif-containing protein 72	TRIM72				-									
Q3MHL4	Adenosylhomocysteinase	AHCY		-											
Q86TD4	Sarcalumenin	SRL					+								
Q0VCG3	Parvalbumin alpha	PVALB					+				+	+	-		
P28801	Glutathione S-transferase P	GSTP1						+							6 times +
P00442	Superoxide dismutase [Cu-Zn]	SOD1		-											5 times -
O77834	Peroxiredoxin 6	PRDX6		-											
Q5E946	Protein/nucleic acid deglycase DJ-1	PARK7		-				+	-						
P54149	Mitochondrial peptide methionine sulfoxide reductase	MSRA	+		+	+									
P38657	Protein disulfide-isomerase A3	PDIA3				-	+								
Q5E9K0	Proteasome subunit beta type-2	PSMB2						+							2 times +
Q5E9F9	26S proteasome regulatory subunit 7	PSMC2							+						
		Total	9	22	10	17	16	8	14	15	9	4	9	3	74 times +
															55 times -
															7 times +/-

[1] The proteins in the rows shown in green are highest in tender meat, those in red are lowest in tender meat and those in orange are both positive and negative depending on the protein isoform.



Highlights

- First meta-proteomics gathering a comprehensive list of candidate biomarkers of beef tenderness.
- Integration of 61 putative biomarkers of beef tenderness from 12 proteomic studies from the same laboratory.
- Biomarkers of *Longissimus thoracis* and *Semitendinosus* muscles were grouped into 6 biological pathways.
- Biological mechanisms underpinning muscle to meat conversion.
- Robust biomarkers under several factors of genders, breeds, muscles and evaluation method of tenderness.
- Hsp27 (HSPB1) is an interesting biomarker of beef tenderness.

Declaration of Competing Interest

Authors declared no conflict of interest.