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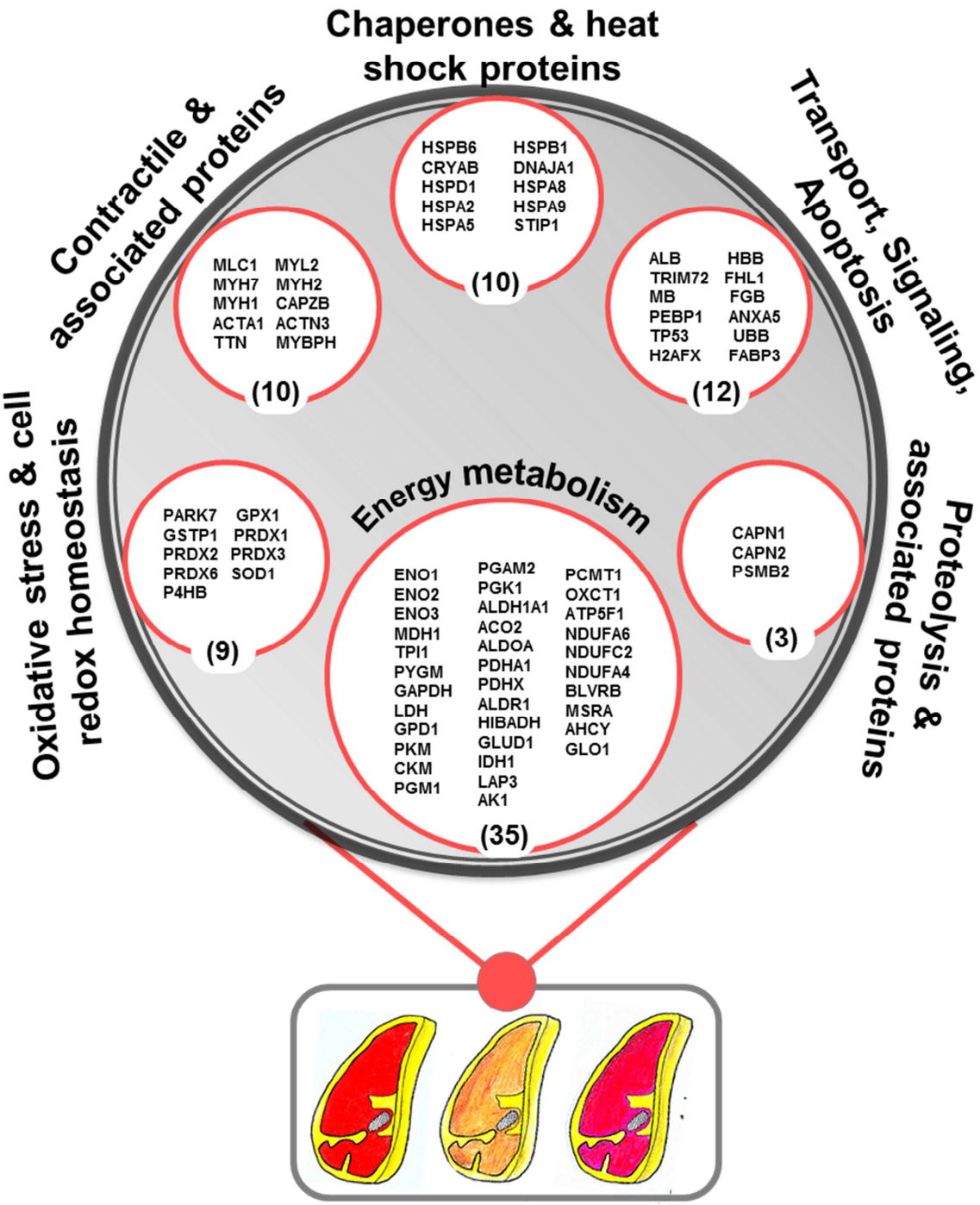

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Abstract

Background: Implementation of proteomics over the last decade has been an important step toward a better understanding of the complex biological systems underlying the conversion of muscle to meat. These sophisticated analytical tools have helped to reveal the biochemical pathways involved in fresh meat colour and have identified key protein biomarkers.

Scope and approach: Until recently, there have been no detailed or critical studies on the role of protein biomarkers in determining meat colour. This review presents an integromics of recent muscle proteomic studies to investigate pathways and mechanisms of beef colour. A database was created from 13 independent proteomic-based studies including data on five muscles and a list of 79 proteins which were significantly correlated with colour traits. The database was subjected to a multistep analysis including Gene Ontology annotations, pathway analysis and literature mining. This report discusses the key protein biomarkers and the biological pathways associated with fresh beef colour. Biomarkers were prioritised by the frequency of identification and the need for future validation experiments is discussed.

Key findings and conclusions: This review identifies six pathways involved in beef colour including energy metabolism, heat shock and oxidative stress, myofibril structure, signalling, proteolysis and apoptosis. The data-mining of the list of the putative biomarkers showed that certain proteins, such as β-enolase (ENO3), Peroxiredoxin 6 (PRDX6), HSP27 (HSPB1), Phosphoglucomutase 1 (PGM1), Superoxide Dismutase (SOD1) and μ-calpain (CAPN1) were consistently reported by multiple studies as being differentially expressed and having a significant role in beef colour. This integromics work proposes a list of 27 putative biomarkers of beef colour for validation using adapted high-throughput methods.

Keywords: Proteomics; Beef colour; Integromics; Biomarkers; Muscle proteome; Biological pathways.

1. Introduction

Meat colour is critical to fresh beef marketability as it influences consumer purchase decisions and attractiveness at the point-of-sale. Historically, the role of muscle proteins in meat colour have been identified including the important role of fibre type (Klont *et al.* 1998), glycolysis and sarcoplasmic proteins (Nair *et al.* 2018b), oxidation and myofibrillar structure (Hughes *et al.* 2014; Gagaoua *et al.* 2017c). During the past two decades, sophisticated –OMICs technologies within a Foodomics approach have been applied by meat scientists to elucidate the biological basis/mechanisms of meat quality traits including colour, with varying success (Nair *et al.* 2017). Proteomics can be an efficient tool to study the dynamic biochemical changes taking place in the *post-mortem* muscle (Jia *et al.* 2007; Nair *et al.* 2018b). Proteomics combined with mass spectrometry (MS) or proteomic-based techniques, were able to offer increased resolving power and capability to separate and identify a great number of muscle proteins, allowing a more in-depth study of the conversion of muscle to meat and associated eating qualities (Picard & Gagaoua 2020).

Proteomics is a quantitative analysis technique involving large-scale and systematic characterization of the whole protein content (proteome) present in a cell, tissue, or organism at given moment and environmental conditions. Proteome analysis depends on five major steps; protein separation, identification, characterization, quantification and functional characterization, allowing the study of interactions between the proteins. The muscle proteome can be studied at the level of proteins or at the peptide level after protein digestion, referred to as “top-down” or “bottom-up” approach, respectively. In the former approach, one- (1DE) or two-dimensional (2DE) gel electrophoresis coupled to MS is the most common technique for both separation and identification of the proteins (Ohlendieck 2011; Picard & Gagaoua 2020). As an alternative to this time-consuming approach, new “bottom-up” versatile and cost-effective MS technologies with much better sensitivity and resolution have been proposed (Moradian *et al.* 2014) and have been recently applied to study meat discoloration and stability (Yu *et al.* 2017a; Yu *et al.* 2017b). These advancements have allowed for the identification of new potential protein biomarkers, which may explain the large variation in, and underlying mechanisms of meat colour other than myoglobin chemistry (Sayd *et al.* 2006; Joseph *et al.* 2012; Gagaoua *et al.* 2017c; Nair *et al.* 2017; Gagaoua *et al.* 2018; Purslow *et al.* 2020). These potential biomarkers have previously been used to explain different meat qualities such as tenderness (Bjarnadottir *et al.* 2012; Picard & Gagaoua 2020), pH (Kwasiborski *et al.* 2008; Huang *et al.* 2011), water-holding capacity (Di Luca *et al.* 2016), marbling and adipose tissue content (Mao *et al.* 2016) as well as protein oxidation and other modifications occurring in *post-mortem* muscle (Lametsch *et al.* 2003).

Proteomics was first used to investigate fresh meat colour in pigs (Hwang 2004) and more recently for beef colour (Kim *et al.* 2008). The proteomic approach can be used to identify the biochemical basis of pre- and post-harvest aspects affecting colour at the point of sale and identify predictive candidate protein biomarkers for colour stability. In view of the vast amount of information generated by subsequent beef colour proteomics trials, and the need for deciphering this information, this integromics work gathers 79 putative protein biomarkers correlated with beef colour traits (lightness (*L**), redness (*a**), yellowness (*b**), among others) irrespective of muscle and proteomic platform. This was generated from published lists of differentially expressed proteins that were significantly correlated with beef colour traits from 13 recent, independent proteomic-based studies. Therefore, this review aims to generate a comprehensive ranked list of candidate biomarkers and attempts to distinguish key candidate beef colour biomarkers from spurious proteins. Consequently, these key biomarkers are discussed in relation to the mechanistic biological processes and pathways involved in beef colour determination and stability.

2. Molecular and structural basis of meat colour and evaluation methods

2.1 Meat colour definition and measurement

The colour of meat is dependent on both the chromatic attributes and on the achromatic (without colour) attributes. Chromatic and achromatic contributions to meat colour can be derived from measurements of reflectance on a meat surface and are described by the absorption (*K*) and scattering (*S*) coefficients, respectively for each attribute, or *K/S* ratio as a combined trait for overall colour perceived (Macdougall 1970). Each attribute contributes to the overall colour of the meat, and together they generate the overall colour perception for the consumer.

Chromatic attributes to meat colour are dominated by the contribution from myoglobin and consequently have received much more research attention, with some excellent reviews published on oxidation rates, pigment quality and myoglobin chemistry (Mancini & Hunt 2005; Faustman *et al.* 2010). In the CIE-*L*a*b** colour space, chromatic attributes are associated with the redness (*a**) and yellowness (*b**) measurements and consequently can also be characterised using Chroma (*C**, saturation index) or hue angle (*h**, specific colour family related to wavelength) (Warner 2014). Myoglobin, is a tetrameric heme protein that contains a centrally located iron atom. The oxidation or oxygenation state of this iron atom and/or the ligand attached can determine the pigment colour. For fresh meat, this colour would be purple, red or brown for deoxy- (DMb), oxy- (OMb) or metmyoglobin (MMb), respectively.

2.2 Colour *versus* colour stability

Colour stability is usually documented by measuring the change in the predominant myoglobin forms over time, for example by sequential measurement of R630/580 over time. Many experiments only measure colour at one fixed time, post-blooming, and in this case, *L**, *a**, *b** are excellent descriptors. But in order to measure colour stability (and hence shelf-life, in regard to colour), the meat samples need to be challenged to express variation in oxidative and colour stability, through time, and/or packaging, including modified-atmosphere packaging. Simulated retail display is often used and repeated measurements of the changes in measures such as R630/580 enables predictions to be made of phenotypic and genetic contributions to the colour stability of meat under simulated retail display (Jacob *et al.* 2014). The metmyoglobin reducing activity (MRA) is an indication of the reduction process of the pigment and provides an additional measure of colour stability.

Achromatic contributions to meat colour are determined by the physical and structural properties of the muscle, and to a lesser extent the contribution of myoglobin, and have received far less research attention than the chromatic contributions. Some scientists, such as Macdougall (1970) and Irie and Swatland (1992), have highlighted the importance of transmittance, reflectance and light scattering in determining the achromatic properties of the meat. In the CIE-*L*a*b** colour space, achromatic attributes are predominantly associated with the *L**. More recently, reflectance confocal laser scanning microscopy has also been used to qualitatively visualise structural characteristics, while quantitatively providing an indication of light scattering (Purslow *et al.* 2020). Combined, these chromatic and achromatic attributes generate the meat colour observed by the consumer.

2.3. Mechanisms by which proteins may influence beef colour

As discussed above, the concentration of myoglobin (protein pigment) in a given muscle determines the redness *a**-value and yellowness *b**-value of the meat surface, and also the *L**-value. As animals mature, their muscles switch to being more aerobic, with higher levels of myoglobin and also of oxidative enzymes, hence the muscle colour changes from the white to pale-pink colour of veal to the bright/deep red colour of mature beef. The concentration of myoglobin also varies between muscles. Hence the darker red colour of the muscles containing predominantly slow-twitch, oxidative type I fibres which contain higher levels of myoglobin (e.g. *Psoas major*) compared to the paler red colour of muscles containing predominantly fast-twitch glycolytic type IIb fibres (e.g. *Longissimus lumborum*) which contain lower levels of myoglobin. Variations in levels of myoglobin, oxidative enzymes and organelles (especially mitochondria) would therefore be expected to be associated with variations in beef colour.

Conversely, the lightness of a meat surface, which is measured by the *L**, is mainly caused by scattering of light from within the muscle structure as well as fluid on the surface (Purslow *et al.* 2020) and to a lesser extent by the concentration of the pigment myoglobin. Both light scattering and surface fluid are generally greater in muscles with predominantly glycolytic fibre types, which have a tendency to exhibit a pale, weepy quality defect called PSE in pork, and which also occurs in beef muscle. These predominantly fast-twitch glycolytic muscles will not only have lower concentrations of myoglobin and associated enzymes, but will also tend towards faster pH fall *post-mortem* and lower ultimate pH, principally due to higher glycogen storage. The glycolytic rate influences *post-mortem* changes to proteins such as myosin, actin, troponin, and some metabolic proteins, particularly glycolytic enzymes in the sarcoplasm, and these *post-mortem* protein changes can influence the ultimate meat quality. Conversely, oxidative slow twitch fibre types generally have lower glycogen storage and tend towards having a high ultimate pH, which is associated with lower *L** values, due to both reduced light scattering (Purslow *et al.* 2020) and higher oxygen consumption in the surface. Thus, variations in levels of glycogen and in glycolytic and oxidative enzymes would also be expected to be associated with variations in beef colour, but through a different mechanism to myoglobin. Differences in contractile and metabolic properties, including variations in pH, certainly contribute to the differences in both colour and colour stability of different muscles, and different regions of muscles (Nair *et al.* 2018a). The principal causes of changes in the achromatic aspects of meat colour are thought to be the lateral spacing of myofilaments/myofibrils and the denaturation of sarcoplasmic proteins.

3. Database creation: literature search strategy, inclusion criteria and data collection

A computerized search using Pubmed.gov (NCBI), Google Scholar, Web of Science (Clarivate Analytics) and Scopus databases was performed, attempting to identify all relevant published proteomics studies dealing with meat colour. Databases were searched from August 2018 to January 2020 for studies published from 2000 to 2020. The keywords used were “proteomic”, “omic”, “proteome”, “protein”, “biomarker” and “colour”, in combination with “meat” or “muscle”. There were no language or data restrictions, but only proteomic studies using muscle samples (meat) were considered. The literature search focused exclusively on full text articles published in peer-reviewed journals to ensure the methodological quality of the studies.

Citations selected from this initial literature search yielded 239 articles which were subsequently screened for eligibility (PRISMA method) to fulfil the objectives of this review and focused on bovine meat only. Therefore, the main inclusion/exclusion criteria were: **i)** proteomics on bovine meat (beef); **ii)** only proteins that were shown in the article to be significantly correlated ($P < 0.05$) with colour traits; and **iii)** colour traits excluding high pH, dark-cutting or DFD (Dark, Firm and

Dry) meat as the mechanisms are different. Thirteen peer-reviewed research articles including those using targeted proteomics (Kim *et al.* 2008; Joseph *et al.* 2012; Canto *et al.* 2015; Gagaoua *et al.* 2015; Wu *et al.* 2015; Nair *et al.* 2016; Wu *et al.* 2016; Gagaoua *et al.* 2017a; Gagaoua *et al.* 2017b; Gagaoua *et al.* 2017c; Yu *et al.* 2017a; Gagaoua *et al.* 2018; Yang *et al.* 2018) fulfilled the criteria and were used to create the database of beef colour biomarkers (**Table 1** and **Table S1**).

4. Database description

The database created includes the following details, for each reference; study number (from 1, the oldest to 13, the newest publication), author's name, publication year, country of the authors, the breed of the animals used and when possible the gender and type, muscle, number of animals included, colour traits/instrument/conditions of measurement and the proteomics platform used (**Table S1**). The data collected included five muscles which are known to differ in their contractile and metabolic properties (Totland & Kryvi 1991) and their colour stability (McKenna *et al.* 2005) being; L, *Longissimus* muscle (mixed oxido-glycolytic, highly colour-stable); RA, *Rectus abdominis* (mixed fast oxido-glycolytic, likely to be colour-labile but not described anywhere); ST, *Semitendinosus* (fast glycolytic, highly colour-stable); SM, *Semimembranosus* (oxido-glycolytic, intermediate colour-stablility) and PM, *Psoas major* (oxidative, colour-labile). These classifications are somewhat generalised. The term *Longissimus* (L) refers in this review to m. *longissimus dorsi*, m. *longissimus lumborum* and m. *longissimus thoracis.*

The portable machines used for colour measurements in the studies were Minolta chromameter (CR-300 and CR-400), HunterLab (labscan, XE or XE plus) and X-rite spectrophotometer handheld devices and measure L^*, a^*, and b^*. In addition, the specifications for light source, observer angle, and aperture either varied or were not given, consistent with the review of Tapp *et al.* (2011).

Different machines, different versions of machines and variation in the aperture size can influence the colour measurements obtained (Warner 2014) although specifying a different illuminant or observer angle within a machine, usually only has a negligible influence. Some of the studies used a^* and b^* values to compute hue angle (h^*) to assess discoloration, and saturation index (C^*) to evaluate red colour intensity (AMSA 2012). Other colour parameters reported in some studies were; MRA, Metmyoglobin Reducing Activity, OCR, Oxygen Consumption Rate and R630/580, surface colour stability. Ten studies that reported protein abundances, but not correlation analyses, or comparisons of muscle proteome abundance with ageing time colour traits or proteomics of dark cutting, were not included in the database (Yu *et al.* 2017b; Mahmood *et al.* 2018; Nair *et al.* 2018b; Zhai *et al.* 2018; Zhang *et al.* 2018; Hughes *et al.* 2019; Kim *et al.* 2019;

Wu *et al.* 2019; Wu *et al.* 2020). However, the results from these studies are cited and discussed where useful in this review.

The 13 articles retrieved and included, were scrutinized and key information regarding the proteins of interest (unique gene names (GN)) and their relationships ($P< 0.05$) with colour traits were annotated by giving their Uniprot ID accession numbers, GN, full name of the proteins and the direction of association (positive or negative) with colour traits (**Table 1**). Within a paper, if the same protein was significantly correlated ($P< 0.05$) with a trait in the same direction more than once due to either different isoforms or detection under different conditions, it was listed only once. Thus, the total number of 79 proteins (unique GN) for the five muscles were identified, several of which were common for several muscles (**Figure 1**).

It should be noted that the selection criteria for proteins identified in the 13 papers differed somewhat; eight papers selected proteins by 1.5-fold, or two-fold, or $P<0.05$ differences in protein abundance between treatments. The remaining five papers analysed the abundance of 21 – 29 pre-selected protein biomarkers that had been identified in earlier studies. The highest number of proteins were identified for the *Longissimus* muscle, due to its more frequent use, with 59 proteins across the various colour traits, and 54 of them correlated with *L**, *a** or *b** (**Figure 1**). The ST muscle followed with 27 proteins (25 correlated with *L**, *a** or *b**), the PM muscle with 19 proteins (17 correlated with *L**, *a** or *b**), the RA muscle with 14 proteins (12 correlated with *L**, *a** or *b**) and the SM muscle with 6 proteins, correlated with only MRA and R630/580 colour traits (**Table 1**). The Venn diagram illustrates the degree of overlap among the muscles by summarising the similarities (common) and divergences (specific) within the proteins for the five muscles and for each muscle (**Figure 1**).

The Venn diagram of **Figure 2A** indicates that 73 of the 79 putative markers identified the specific muscle and breed/animal type which were correlated to the coordinates *L** (25 proteins of which 3 specific), *a** (66 proteins of which 43 specific) and *b** (24 proteins of which 2 specific). Seventeen proteins were common to the three colour coordinates: HSPB6, HSPB1, CRYAB, DNAJA1, HSPA8, HSPA2, HSPA9, ENO3, MDH1, PGM1, PRDX6, MYH7, MYH2, MYH1, ACTA1, TTN and CAPN1. Within the *Longissimus* muscle, 54 out of 59 proteins were correlated to the coordinates *L** (23 proteins with 3 specific), *a** (45 proteins with 25 specific) and *b** (23 proteins with 2 specific). Thirteen proteins were common to all three coordinates: HSPB6, HSPB1, CRYAB, DNAJA1, HSPA8, HSPA2, HSPA9, ENO3, MDH1, PRDX6, MYH2, ACTA1, and TTN (**Figure 2B**).

5. Mining the putative protein biomarkers of beef colour – database analysis

A computational workflow allowed aggregation of the data from the 13 publications and creation of the first list of putative protein biomarkers of beef colour that was subsequently mined using web service bioinformatics tools. The list of 79 proteins was submitted to a custom analysis using ProteINSIDE (http://www.proteinside.org/). ProteINSIDE obtains results from several software and databases with a single query (Kaspric *et al.* 2015). Using this tool, Gene Ontology (GO) enrichment tests (*P*-value, Benjamini Hochberg < 0.05) were also performed using human orthologs to take advantage of the most complete annotation available (**Figure S1 and Table S2**). The ProteINSIDE tool relies mainly on GO enrichment tests among the Biological Process (BP), Molecular Function (MF) and Cellular Component (CC) categories.

The protein-protein interactions (interactomics) of biological function of the proteins from the five muscles (**Figure 3A**) or LT muscle alone (**Figure 3B**) were analysed using the 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. Considering the limitation of the GO annotation of genes in bovine, we converted their UniprotIDs to orthologous human EntrezGene IDs using BioMart (http://www.ensembl.org/biomart/). Functional annotation analysis of the 79 proteins was further checked using the PANTHER classification system to identify ontology categories for the complete list of proteins and to identify the main biological pathways. Among the 79 proteins we further searched for secreted proteins that may be secreted in the extracellular environment and involved in interactions between cells at short and long distances. Eight of such proteins (HSPB6, HSPA5, GAPDH, PARK7, PRDX6, P4HB, ALB and FGB) were revealed by ProteINSIDE tool and were highlighted in the protein networks (**Figure 3A,B**) by black and red ovals, for those secreted by classical pathways or non-classical pathways, respectively.

6. Six Main biological pathways associated with beef colour

The GO analyses showed that the 79 proteins clustered into 6 distinct but strongly interconnected biological pathways (**Table 1, Figure S2** and **Figure S3**). These pathways are known to be related to beef tenderness (Guillemin *et al.* 2011; Ouali *et al.* 2013; Picard & Gagaoua 2020). This suggests that the biological pathways associated with variations in meat tenderness and meat colour are related. The main pathways and related proteins are (see **Table 1** for full names of each protein):

i) Chaperones & heat shock proteins: HSPB6, HSPB1, CRYAB, DNAJA1, HSPD1, HSPA8, HSPA2, HSPA9, HSPA5 and STIP1.

ii) Catalytic, metabolism & ATP metabolic process (this pathway was organised into 3 main sub-pathways and all the proteins are shown in **Figure 4**):

a. Glycolysis and associated pathways: PYGM, PGM1, ALDOA, TPI1, GAPDH, PGK1, PGAM2, ENO1, ENO2, ENO3, PKM2, LDH, PDHA1, PDHX, GPD1 (n = 15).

b. Tricarboxylic acid cycle and associated pathways: MDH1, ACO2, HIBADH, GLUD1, IDH1, OXCT1, ATP5F1, NDUFA6, NDUFC2, NDUFA4 (n = 10).

c. Other catalytic and ATP metabolic pathways grouping oxidoreductase, transferase, hydrolase, lyase & kinase: CKM, ALDH1A1, ALDR1, LAP3, AK1, PCMT1, BLVRB, MSRA, AHCY, GLO1 (n = 10).

iii) Oxidative stress & cell redox homeostasis: PARK7, GPX1, GSTP1, PRDX1, PRDX2, PRDX3, PRDX6, SOD1 and P4HB.

iv) Contractile & associated proteins: MLC1, MYL2, MYH7, MYH2, MYH1, CAPZB, ACTA1, ACTN3, TTN and MYBPH.

v) Proteolysis & associated proteins: CAPN1, CAPN2 and PSMB2.

vi) Binding, cofactor & transport proteins, signalling or apoptosis: ALB, HBB, TRIM72, FHL1, MB, FGB, PEBP1, ANXA5, TP53, UBB, H2AFX and FABP3.

The distributions and molecular functions of the clustered groups of proteins of interest are described for each muscle in **Table S4** and **Table S5**. The presence/absence of these proteins in the 13 studies is illustrated in **Figure S3**. Across the muscles, the most represented functions for the five muscles taken together are: proteins involved in catalytic, metabolism and ATP metabolic processes (44.3%, 35 proteins); binding, cofactor and transport proteins, signalling or apoptosis (15.2%, 12 proteins); chaperones and heat shock proteins (12.7%, 10 proteins) and contractile and associated proteins (12.7%, 10 proteins). Oxidative stress and cell redox homeostasis, and proteolysis and associated proteins, represent 11.4% (9 proteins) and 3.8% (3 proteins), respectively. Hence; irrespective of the muscle, the catalytic, metabolism and ATP metabolic process pathway dominates (**Table S5**). Of the five muscles, *Longissimus* muscle was considered representative (more interesting), and was chosen to illustrate the functional interaction network (**Figure 5**). The lack of identified biological pathways in some muscles is probably due to low numbers of identified proteins due to the lower representation of some muscles across the studies.

7. Putative protein biomarkers most frequently correlated with beef colour traits

Of the 79 protein biomarkers, 27 were reported 3 to 8 times in independent studies, generally as being correlated with beef colour traits. These similarities ranged from one protein common to 8 studies to 13 proteins identified in at least 3 studies (**Table 1** and **Figure S3**). At the top of this list, β-enolase (ENO3) was correlated with colour traits in 8 studies (Joseph *et al.* 2012; Gagaoua *et al.* 2015; Nair *et al.* 2016; Wu *et al.* 2016; Gagaoua *et al.* 2017a; Gagaoua *et al.* 2017b; Gagaoua *et al.* 2017c; Yu *et al.* 2017b); Peroxiredoxin 6 (PRDX6) (also found as a secreted protein) in 7 studies (Gagaoua *et al.* 2015; Wu *et al.* 2015; Wu *et al.* 2016; Gagaoua *et al.* 2017a; Gagaoua *et al.* 2017b; Gagaoua *et al.* 2017c; Yang *et al.* 2018); HSP27 (HSPB1, also identified as a secreted protein) in 6 studies (Kim et al. 2008; Joseph et al. 2012; Wu et al. 2016; Gagaoua et al. 2017a; Gagaoua et al. 2017b; Gagaoua et al. 2017c); and 5 studies found that Phosphoglucomutase 1 (PGM1), Superoxide Dismutase (SOD1) and Calpain-1 catalytic subunit or μ-calpain (CAPN1) were related to colour (**Table 1** and **Figure S3**). Eight other proteins: HSP40 (DNAJA1), HSP70-8 (HSPA8), HSP70-Grp75 (HSPA9), Malate dehydrogenase 1 (MDH1), Triosephosphate isomerase 1 (TPI1), Lactate dehydrogenase (LDH), Myosin-7 (MYH7) and Myosin-2 (MYH2) were reported 4 times and 13 were reported 3 times: HSP20 (HSPB6), αB-crystallin (CRYAB), HSP72 (HSPA2), Pyruvate kinase M 2 (PKM), Creatine kinase M type (CKM), Fructose-bisphosphate aldolase A (ALDOA), DJ-1 (PARK7), Peroxiredoxin 2 (PRDX2), Myosin light chain 1 (MLC1), Myosin-1 (MYH1), α-Actin, (ACTA1), Myosin binding protein-H (MYBPH) and Phosphatidylethanolamine-binding protein 1 (PEBP1).

Of these 27 proteins, 8 belong to the catalytic, metabolism and ATP metabolic pathway; 7 were chaperones and heat shock proteins; 6 were contractile and associated proteins; 4 belong to the oxidative stress and cell redox homeostasis pathway and one protein for each of the two other pathways. In the following sections, we discuss the biochemical mechanisms which may underlie the relationships between these proteins and beef colour.

7.1. Protein biomarkers belonging to the catalytic, metabolism & ATP metabolic pathway

Among the 8 metabolic enzymes, 6 are glycolytic (ENO3, PGM1, TPI1, LDH, PKM, and ALDOA) shared between the preparatory and energy-yielding phases (**Figure 4A**), hence indicating the importance of this pathway in beef colour determination. Of the two other proteins, one belongs to the Krebs cycle (MDH1, an oxidative enzyme) and the other, CKM, is the initial pathway used for ATP regeneration by creatine phosphate, before the switch to ATP generation via the glycolytic pathway (**Figure 4B,C**). The direction of correlation between these proteins and colour attributes differs according to the muscle and the colour parameter (**Table 1** and **Figure 5**). Inversion of

correlations occurs when the biochemical mechanism underlying the correlation has a major contribution to the studied phenomenon but other factors are also involved. The multifactorial and interdependent character of the influence of *post-mortem* metabolism and oxidative properties on meat colour is well known and may explain the inversion of correlations observed. For example, lactate, produced under the anaerobic conditions of glycogen breakdown in the *post-mortem* muscle, may influence the redox status of the muscle, which may impact meat colour (Joseph et al. 2012). Regeneration of ATP depends on glycogen breakdown and is correlated to the extent of pH decline (Robergs *et al.* 2004), which can also influence colour, both from a myoglobin status and from a structural perspective. Metabolism of ATP is the key determinant of the rate and extent of pH fall *post-mortem* which, through its effect of decreasing myofilament lattice spacing and myofibril diameter, has a contribution to increasing values of *L** through the achromatic processes of light scatter (Hughes *et al.* 2019). Some proteins of this group reported in **Table 1**, highlight that an increase in protein abundance is associated with a decrease in *L**. This observation suggests that the increased amounts of some glycolytic enzymes are largely not affecting *L** values through this achromatic mechanism.

7.1.1. ENO3, a robust biomarker irrespective of muscle and colour parameter

Enolase is a cytosolic enzyme involved in an intermediate step in glycolysis and responsible for the conversion of 2-phosphoglycerate into phosphoenolpyruvate (**Figure 4**). It is an important moonlighting enzyme that is associated with stress and hypoxic conditions and in some species has been shown to be over-expressed under acidic conditions (Didiasova *et al.* 2019). Enolase is a dimer existing in 3 isoforms, ENO1 (formed from two α subunits), ENO2 (formed from two γ subunits) and ENO3, formed from two β subunits. The ENO3 isoform seems to play an important role in beef colour (8 studies); ENO1 and ENO2 were only found together in one study, where they were negatively correlated in *Longissimus* muscle (Gagaoua *et al.* 2018) and in the ST muscle, respectively (Yu et al. 2017a). In beef, ENO3 is the isoform related to tenderness (Ouali *et al.* 2013; Picard & Gagaoua 2020) explained by the fact that ENO3 is predominantly found in striated muscles, whereas the other two isoforms are located elsewhere: ENO1 in the embryonic form of all tissues and ENO2 in the neuron and neuroendocrine tissues.

The consistent appearance of ENO3 in the lists of proteins that vary significantly with both meat colour and colour stability points to the importance of this glycolytic enzyme. This may be related to its role in glucose metabolism under hypoxic conditions (Sedoris *et al.* 2010) and cellular protection during hypoxia (Wulff *et al.* 2012). Depending on the study, correlations between the abundance of ENO3 and *L**, *a**, *b** and MRA are positive or negative suggesting complex interactions between this step in glycolysis and other mechanisms affecting colour. For example,

ENO3 participates in multi-enzyme complexes present on the sarcomere, possibly in association with other proteins (**Figure 3A,B**) involved in colour, such as heat shock proteins (Wulff et al. 2012) and contractile proteins (Hughes *et al.* 2014; Hughes *et al.* 2019). In keeping with this, Nair *et al.* (2018a) comparing the inside colour-labile and the outside colour-stable regions of the *Semimembranosus* (SM) muscle, found differences in the sarcoplasmic proteome, including ENO3. They suggest that differences in the contractile and metabolic properties including variations in pH, contribute to the differences in colour stability of the two regions (Nair et al. 2018a).

7.1.2. PGM1, TPI1, LDH and PKM are glycolytic enzymes and are biomarkers for beef meat colour, irrespective of the contractile and metabolic properties and colour-stability of muscles

Phosphoglucomutase 1 (PGM1), is an enzyme playing a central role in glycolysis and glycogenesis, reversibly catalysing the conversion of glucose-1-phosphate (G-1-P) to glucose-6-phosphate (G-6-P) (**Figure 4**). PGM1 was correlated with colour traits in 4 muscles (**Figure 5A**) from 5 studies (Canto et al. 2015; Wu et al. 2016; Gagaoua et al. 2017a; Gagaoua et al. 2017b; Yu et al. 2017a). Correlations were negative in ST and PM, and positive in RA. Considering the *Longissimus* muscle in detail, PGM1 was positively related to *L** and, depending on the study, positively or negatively with *a**-values (**Figure 5B**).

The relationships between PGM1 and several colour traits from different muscles and animal types are consistent with the knowledge that glycolysis during early *post-mortem* period affects many meat quality properties including tenderness and colour (Anderson *et al.* 2014). First, hypoxic conditions occurring in the *post-mortem* muscle, increase the abundance and the activity of PGM1, which at least partly regulates the *post-mortem* balance between G-1-P and G-6-P. The latter may be associated to meat colour, through the effect on pH decline. For example, metabolite profiles, including G-6-P in samples collected during the early *post-mortem* period (Yu *et al.* 2019) differed amongst muscles which were subsequently found to differ in colour stability during display (Abraham *et al.* 2017). Second, PGM1 undergoes posttranslational modifications through phosphorylation, acetylation or methylation (Anderson et al. 2014) and the direction of associations of PGM1 with various colour parameters could be influenced by its isoforms (Anderson *et al.* 2014). Earlier studies suggested that according to muscle, stress level of the animal or the use of *post-mortem* electrical stimulation, different PGM1 isoforms correlate with different meat quality indicators (Laville *et al.* 2009; Bjarnadottir *et al.* 2010; Anderson *et al.* 2014). At relatively high levels of G-1-P, greater activity of PGM1 increases the rate of its conversion into G-6-P, which, depending on the energy requirements of the cell is either used in the glycolytic pathway, or for regeneration of NADH; the latter being an important determinant of meat colour stability (Mancini & Hunt 2005; Mitacek *et al.* 2019). Hence, depending on the G-1-P and G-6-P balance, the levels of

posttranslational modifications of PGM1 and the cellular requirements for ATP, greater PGM1 activity may favour better colour stability of the meat.

Triosephosphate isomerase (TPI1) was common to 4 studies (Wu *et al.* 2015; Nair *et al.* 2016; Wu *et al.* 2016; Gagaoua *et al.* 2018) and 4 muscles (**Table 1** and **Figure 1**). The relationships were negative for ST and PM muscles, and in different directions for *Longissimus* muscle (negative with *a** and positive with *b**). In addition, it had a positive relationship for the SM muscle (**Figure 5**). TPI1 catalyses the reversible conversion of dihydroxyacetone phosphate to D-glyceraldehyde 3-phosphate (**Figure 4**) completing the splitting stage in first preparatory phase of glycolysis. TPI1 abundance, including its phosphorylated isoforms, were positively related to the rate of pH decline, indicating its role in *post-mortem* glycolysis and may partly explain its association with meat colour (Huang *et al.* 2011; Gagaoua *et al.* 2018; Nair *et al.* 2018a; Wu *et al.* 2019). TPI1 has also previously been associated with beef tenderness (Grabez *et al.* 2015; Picard & Gagaoua 2020).

Lactate dehydrogenase (LDH) was significantly correlated with colour traits in 4 studies, involving three muscles (Wu *et al.* 2015; Gagaoua *et al.* 2017a; Gagaoua *et al.* 2017b; Gagaoua *et al.* 2017c). Specifically, LDH was positively related to colour parameters in the RA and ST muscles (**Figure 5**) and in both directions in *Longissimus* (negative with *L** and positive with *a**). LDH catalyzes the reversible conversion of pyruvate to lactate with the conversion of NADH to NAD+ (**Figure 4**). NADH is known to promote metmyoglobin reduction, with lower levels of NADH and higher levels of NAD+ favouring metmyoglobin formation (Mancini & Hunt 2005). Therefore, the specific role that LDH may play in metmyoglobin reduction is important to understand the consequences of colour stability of different bovine muscles.

Pyruvate kinase M 2 (PKM) has four isoforms, two of which, PKM1 and PKM2, predominate in skeletal muscle . In three of the studies (Canto *et al.* 2015; Wu *et al.* 2015; Wu *et al.* 2016) PKM2 was negatively correlated with colour parameters in the ST and PM muscles, similar to the TPI1 and PGM1 enzymes, and, depending on the muscle, in different directions for *Longissimus* muscle for *a**, MRA and R630/580 parameters (**Table 1** and **Figure 5**). PKM catalyzes the last step of glycolysis, the dephosphorylation of phosphoenolpyruvate to pyruvate and a greater PKM abundance is likely to reflect a potentially greater production of pyruvate (**Figure 4**). Pyruvate is transported into the mitochondria and favours the regeneration of NADH (Ramanathan & Mancini 2010). Muscle type and many other factors influence the glycolytic capacity of muscles which is consistent with the differences obtained between studies (**Table S1**).

7.1.3. ALDOA, a negative biomarker in colour-stable beef muscles

Fructose-bisphosphate aldolase A (ALDOA) was related to colour parameters in 3 of the studies (Wu *et al.* 2015; Nair *et al.* 2016; Wu *et al.* 2016) being negatively correlated with *a** and MRA colour traits for the *Longissimus*, SM and ST muscles (**Table 1** and **Figure 1**). During glycolysis, ALDOA catalyses the conversion of fructose 1, 6-diphosphate to glyceraldehyde 3-phosphate (**Figure 4**). Its negative association with colour parameters might partly be explained by differences in muscle fibre type composition. Higher levels of ALDOA may be indicative of increased proportions of fast-twitch muscle fibres, hence lower oxidative metabolism and higher glycolytic metabolism. Any difference in oxygen consumption may lead to increased formation of oxymyoglobin and to redder beef (high *a**-values) and reduced metmyoglobin (high MRA). Accordingly, ALDOA has been shown to boost glycolysis upon phosphoinositide 3-kinase (PI3K)/Akt signalling pathway (Hu *et al.* 2016). The redistribution of ALDOA in response to PI3K signalling needs coordinated action between cytoskeletal dynamics and glycolysis. According to Hu *et al.* (2016), a number of glycolytic enzymes are associated with the cytoskeleton, which presumably are released when actin dynamics increase during apoptosis onset, and the change of glycolytic flux appears to be primarily mediated by the mobilization of ALDOA. This is in agreement with Hughes *et al.* (2019) who found that ALDOA affects beef colour by influencing light scattering of muscle fibres. In association with other metabolic enzymes, ALDOA assists in the creation of cross links between adjacent actin filaments or in binding troponin to the thin filaments, hence affecting the distance between myofibrils and therefore light scattering (Hughes *et al.* 2019).

7.1.4. CKM, a positive biomarker in colour-stable beef muscles

Creatine kinase M type (CKM) was correlated positively with *a**, MRA and R630/580 in the *Longissimus* and SM muscles in 3 studies (Joseph et al. 2012; Nair et al. 2016; Yang et al. 2018). CKM was also reported in other proteomic studies to play an important role in beef colour (Mahmood et al. 2018; Nair et al. 2018a; Zhai et al. 2018). This protein has been described as a biomarker of most quality traits of meat including tenderness, drip loss, water-holding capacity and pH decline (Ouali *et al.* 2013). In striated muscles, CKM allows the transfer of a phosphate group from phosphocreatine to ADP to generate ATP (**Figure 4**). During the first hours *post-mortem*, CKM allows the maintenance of ATP regeneration, without a net production of hydrogen ions (Robergs et al. 2004), and therefore is influential in the rate of pH decline (Ouali et al. 2013). During meat ageing, CKM is progressively fragmented and becomes inactive (Jia *et al.* 2007; Laville *et al.* 2009; Bjarnadottir *et al.* 2010). The rate of fragmentation influences the rate of energy depletion and pH decline, explaining its association to the variability of several eating qualities of

beef. In addition, creatine has antioxidant properties (Sestili *et al.* 2011), which could lower myoglobin oxidation in fresh beef.

7.1.5. MDH1, an oxidative enzyme biomarker of colour-stable beef muscles

Malate dehydrogenase 1 (MDH1) was correlated with MRA, *L**, *a**, *b** and *C** parameters in four experiments (Gagaoua *et al.* 2015; Wu *et al.* 2015; Wu *et al.* 2016; Gagaoua *et al.* 2018), in the *Longissimus* and ST muscles (**Table 1** and **Figure 5)**. MDH1 plays an important role in the malate–aspartate shuttle operating between the cytosol and mitochondria. MDH1 also uses the reduction of NAD^+ to NADH to reversibly catalyse the oxidation of malate to oxaloacetate, in the last step of the TCA cycle. The NADH generated can be used in the electron transport chain for maximal ATP production. Thus, variations in MDH1 activity and content may be indicative of differences in the oxidative phosphorylation capacity of the muscle and thus of the variation in beef colour (Gagaoua *et al.* 2015).

7.2. Protein biomarkers belonging to chaperones & heat shock proteins (HSPs)

After the glycolytic enzymes, the second most important family of biomarkers for beef colour, in terms of number and size of correlations, are the heat shock proteins (HSPs). The production of HSPs is generally increased in cells undergoing stress, which is the case of the *post-mortem* muscle in which a variety of perturbations disturbs the *ante-mortem* homeostatic set points. Many HSPs are chaperones that stabilise and ensure correct folding of newly synthesised proteins or help refold proteins altered by cell stress. Ubiquitin, which has many characteristics of a small (8kDa) HSP, cooperates with chaperones to control protein functionality by labelling damaged and misfolded proteins for degradation via the proteasome (Esser *et al.* 2004). A considerable amount of proteasome activity is retained in muscle up to 7 days *post-mortem* (Lamare *et al.* 2002), so that the potential for substantial interaction between HSP activity and proteolysis exists, as discussed in recent studies (de Oliveira *et al.* 2019).

HSPs have been proposed as regulators of apoptosis onset during the conversion of muscle to meat (Sayd *et al.* 2006), consequently affecting proteolysis and meat qualities (Lomiwes *et al.* 2014a; Lomiwes *et al.* 2014b; Picard & Gagaoua 2020). Three small HSPs (HSPB1, HSPB6 and CRYAB), one co-chaperone (DNAJA1) and 3 large 70kDa HSPs (HSPA8, HSPA9 and HSPA2) were identified as major putative markers of beef colour (**Table 1** and **Figure 5**). Other studies on beef colour proteomics also identified these same HSPs to be associated with colour in beef (Yu *et al.* 2017b; Mahmood *et al.* 2018; Zhang *et al.* 2018; Kim *et al.* 2019; Wu *et al.* 2019; Wu *et al.* 2020).

HSPs are often related to sensory meat qualities, irrespective of species, probably because of their multiple roles in the underlying processes (Ouali *et al.* 2013). These processes involve i) regulation of caspases activities; ii) protection of cellular structures against apoptosis; iii) myofibrillar protein protection from degradation by inhibition of proteolytic activity, as discussed above; iv) refolding of denatured proteins caused by pH decline and resultant altered protease activity; and last but not least v) maintenance of the correct conformation of proteins and preservation of their biological functions (Pulford *et al.* 2008; Ouali *et al.* 2013). The following sections focus on features that deserve particular attention.

7.2.1. HSPB1 is the top biomarker of beef colour among the small HSPs

HSP27 (HSPB1) is the only small HSP correlated to colour traits (*L**, *a**, *b**, *C** and R630/580) in two muscles (*Longissimus* and RA) across 6 studies (Kim et al. 2008; Joseph et al. 2012; Wu et al. 2016; Gagaoua et al. 2017a; Gagaoua et al. 2017b; Gagaoua et al. 2017c). The correlations with all colour traits were positive in the RA muscle and in different directions in the *Longissimus* muscle depending on the colour trait and animal type/breed (**Table 1** and **Figure 5**). Two other studies also reported this protein to be related to beef colour (Mahmood et al. 2018; Kim et al. 2019).

The positive and negative relationships observed in the *Longissimus* may be related to differences in the pre-slaughter stress level of the animal and/or the type of HSP27 isoform involved (Ouali *et al.* 2013; Picard & Gagaoua 2020). In skeletal muscle, HSPB1 as well as HSPB6 and CRYAB protect against ischemia, hypertensive stress, and metabolic dysfunction (Dreiza *et al.* 2010). They have the capacity to bind to myofibrils (Lomiwes *et al.* 2014b), thereby protecting skeletal muscle through structural protein complexes. For example, increased CRYAB levels were associated with delayed myofibril degradation in beef with ultimate pH < 5.7. Their role in protection against stress-induced denaturation of muscle proteins may partly explain their relationship with meat colour (Gagaoua *et al.* 2015; Gagaoua *et al.* 2017c). Particularly, the prevention of denaturation of sarcoplasmic proteins and myosin, would affect reflectance, light scattering and myoglobin, thereby influencing all colour coordinates (Hughes *et al.* 2014; Hughes *et al.* 2019; Purslow *et al.* 2020). For example, through their action, the reduction of light scattering would result in a lower *L** as developed in the following sub-section. Other effects may be related to their ability to influence the redox status of the cell. For example, an increase in HSPs content has been associated with lower oxidative stress levels of the cells, as indicated by lower levels of thiobarbituric acid reactive species (Jammes *et al.* 2009).

7.2.2. Specific negative associations of HSPs with Lightness (L) in Longissimus muscle*

The 7 HSPs biomarkers mentioned above were correlated with *L**, *a** and *b** parameters in *Longissimus* muscle (**Figure 5**), and all correlations with *L** were negative. However, they were in different directions for *a** (negative for HSPB1 and HSPB6, positive for CRYAB, DNAJA1 and HSPA9, and different directions for HSPA8 and HSPA2) and *b** (different directions apart from HSPB6, which was always positive) (**Figure 5B**). The consistently negative associations between HSPs and *L** in *Longissimus* muscle are partly explained by their high expression in this muscle (Guillemin *et al.* 2011). As indicated above, their relationship with *L** may be explained by their protective action on structural proteins (Pulford et al. 2008; Lomiwes et al. 2014a; Lomiwes et al. 2014b), including under conditions of apoptosis. Recently, HSPs have also been implicated in dark cutting beef (Mahmood et al. 2018). HSPs appear thus to play a major role in colour development at least through their protective action against protein denaturation, affecting reflectance and light scattering and other aspects of beef colour.

7.3. Protein biomarkers belonging to contractile & associated proteins

Ten myofibrillar proteins, in the contractile and associated proteins group, were correlated to colour traits, 6 of which were shortlisted based on the criteria described above. Most of them were myosin proteins/subunits (MYH1, MYH2, MYH7 and MLC1), myosin-binding proteins (MYBPH) or actin (ACTA1) (**Table 1** and **Figure S3**). The proteolytic degradation of myosin heavy and light chains, binding proteins and actin plays a central role in muscle to meat conversion (Huff-Lonergan *et al.* 2010). During proteolysis, these myofibril proteins are degraded by endogenous muscle proteases, including μ-calpain (see section 7.5), cathepsins and caspases (Ouali *et al.* 2013). Ultrastructural changes in the muscle take place as a result of proteolytic degradation and influence not only the meat texture, but also colour aspects, partly due to the effects on the light scattering arising from the structural elements (Hughes *et al.* 2014). The extent of the structural proteins denaturation and degradation during the *post-mortem* process is influenced by the rigor temperature, and rate and extent of pH decline, and affects the protein density along the sarcomere. For example, myosin degradation influences further myofilament lattice spacing and muscle fibre shrinkage, which impacts also light scattering.

The correlation of the 3 myosin heavy chain isoforms with colour traits may further reflect the role of metabolic enzymes in colour determination. Variations in the abundance of myosin isoforms, glycolytic enzymes and proteolysis are highly interconnected. For example, red, slow-twitch, fibres have greater amounts of mitochondria, enzyme systems allowing oxygen consumption and electron transport chain located in mitochondria (Ramanathan & Mancini 2018; Mitacek *et al.*

2019). Meat colour depends strongly on glycolytic activity, oxygen consumption and reductive enzyme activity in the *post-mortem* muscle. Muscle fibre type proportions differ across muscles and breeds and this may explain the different directions found for the correlations. In addition to differences in enzymes and associated mitochondrial oxygen consumption, reducing capacity through enzymatic and non-enzymatic mechanisms, different muscle fibre types also contain different amounts of pigment other than myoglobin, of glycogen and lipids, which may also influence meat colour (Klont *et al.* 1998; Ramanathan & Mancini 2018). Furthermore, the amount of free water and structural modifications caused by the proteolytic processes following apoptosis and consequently, light reflectance and scattering properties of the meat differ according to fibre type (Hughes *et al.* 2014; Purslow *et al.* 2020). Hence, the complex relationship between myofibrillar proteins and colour are explained by the many direct (myofibrillar protection *versus* degradation) and indirect (differences in properties of the different muscle fibre types and degree of interconnectedness with other pathways) interactions (**Figure 3A,B**).

7.4. Protein biomarkers belonging to oxidative stress & cell redox homeostasis

Skeletal muscles are major oxygen-consuming tissues, characterized by a high rate of mitochondrial respiration, hence presenting an elevated risk of reactive oxygen species (ROS) production. The production of ROS in *post-mortem* muscle has been described as a pivotal event during muscle-to-meat conversion including a major determinant of surface colour, by influencing both the lipid and protein fractions (Ouali *et al.* 2013; Sierra & Olivan 2013). Several protective and endogenous scavenger agents defend the cell against oxidative stress and play a role in meat colour. The present review identified peroxiredoxins (PRDX1, 2, 3, and 6), superoxide dismutase (SOD1), DJ-1 (encoded by *PARK7* gene) and the thioredoxin system, as determinants of meat colour, particularly colour stability, which may be explained by their ability to scavenge ROS and reduce oxidized proteins by means of their active site and the glutathione system.

7.4.1. Peroxiredoxins are potential biomarkers of beef colour

Peroxiredoxin 6 (PRDX6) belongs to the peroxiredoxin family and is a unique member (Fisher 2017), because it has three enzymatic activities being; peroxidase, phospholipase A2 (PLA2) and acyl transferase activity. PRDX6 was the second top shortlisted and important putative biomarker of beef colour in this review. It was identified as a secreted protein (**Figure 3A,B**) and significant in 7 of the studies (Gagaoua *et al.* 2015; Wu *et al.* 2015; Wu *et al.* 2016; Gagaoua *et al.* 2017a; Gagaoua *et al.* 2017b; Gagaoua *et al.* 2017c; Yang *et al.* 2018) and in 4 muscles (**Table 1** and **Figure 1**). Similar to PGM1, PRDX6 was negatively correlated with colour traits for the ST and PM muscles, in both directions for the *Longissimus* muscle (negative with *L** and *b** and positive with *a**) and

positive for the RA muscle (**Figure 5**). The PLA2 (phospholipase A2) of PRDX6 is believed to be activated during cellular stress and triggers ROS production; the enhanced oxidative activity increases contractile function (Gong *et al.* 2006). The positive relationship between Peroxiredoxins and redness, shown by the positive correlations between PRDX6, PRDX1, PRDX2 and PRDX3 and *a**-values in the *Longissimus* muscle, may be related to their different functions. First, they may act as antioxidants, protecting OxyMb from attacks by peroxides (Wang *et al.* 2003). Second, peroxiredoxins may inhibit lipid oxidation, and consequently myoglobin oxidation (Faustman *et al.* 2010; Joseph *et al.* 2012). Finally, PRDX6 may counteract processes underlying meat discoloration by competing with myoglobin for oxygen or products of oxidative stress reactions. PRDX6 is further implicated in a variety of cellular processes, including metabolism, apoptosis and ageing (Fisher 2017) and was proposed as a potential biomarker of beef tenderness (Jia *et al.* 2009; Guillemin *et al.* 2011; Picard & Gagaoua 2020).

The other protein shortlisted, PRDX2, was correlated in opposite directions for the *Longissimus* (positive, colour-stable) and PM (negative, colour-labile) muscles in 3 studies (Joseph et al. 2012; Wu et al. 2016; Yang et al. 2018). PRDX2 has also been identified as a candidate biomarker responsible for meat tenderness (Jia *et al.* 2007; Malheiros *et al.* 2019) and as a main indicator of oxidative stress (Won *et al.* 2012). The mechanisms probably involve the anti-oxidative properties of the protein.

7.4.2. SOD1, a biomarker regardless of the colour-stability of muscles

Superoxide dismutase (SOD1), was correlated in 5 studies with meat colour traits (Gagaoua *et al.* 2015; Wu *et al.* 2016; Gagaoua *et al.* 2017b; Gagaoua *et al.* 2017c; Gagaoua *et al.* 2018) and in 2 muscles (**Table 1** and **Figure 1**). Correlations were positive for the PM muscle and in the opposite direction for *a** and *b** colour coordinates of the *Longissimus* muscle (**Figure 5**). SOD1 is a member of a ubiquitous family of metalloenzymes that eliminate excess ROS, thus preventing damage caused by free radicals in cells (Vacek *et al.* 2010). It allows fast dismutation of O_2^- to O_2 and H_2O_2. SOD1 has been identified as a biomarker of meat toughness (Grabez *et al.* 2015; Malheiros *et al.* 2019; Picard & Gagaoua 2020).

7.4.3. DJ-1 (PARK7), a negative biomarker of colour-stable beef muscles

DJ-1 (also called PARK7), another secreted protein related to oxidative stress, was negatively correlated with *a**, MRA and *C** in the *Longissimus* and PM muscles in 3 studies, irrespective of animal type or breed (Wu *et al.* 2015; Gagaoua *et al.* 2017c; Yang *et al.* 2018). Other studies also reported DJ-1 to be a meat colour biomarker (Mahmood et al. 2018; Nair et al. 2018a). Studies on pork identified DJ-1 as a negative marker of *a** (Sayd et al. 2006) and *L** (Kwasiborski et al. 2008).

DJ-1 has a key role in scavenging mitochondrial H_2O_2 and limiting mitochondrial fragmentation. During oxidative stress, DJ-1 is re-localized from the cytosol to the mitochondria (Junn *et al.* 2009). As indicated above, due to their role in oxygen consumption and their reducing capacity, mitochondria play a major role in meat colour (Ramanathan & Mancini 2018). It is therefore likely that DJ-1 plays a role in meat colour via mitochondria protection, probably by interacting with other pathways, particularly heat shock proteins (Wu *et al.* 2020) and energy metabolism (Picard & Gagaoua 2020). DJ-1 has also been shown to be related to beef tenderness (Jia *et al.* 2007; Jia *et al.* 2009; Mahmood *et al.* 2018; Malheiros *et al.* 2019; Picard & Gagaoua 2020).

7.5. CAPN1 and PEBP1 as protein biomarkers of beef colour

The proteolytic enzyme μ-calpain (CAPN1) and phosphatidylethanolamine-binding protein 1 (PEBP1) were related to colour in 5 and 3 studies, respectively (**Table 1** and **Figure S3**). The role of CAPN1 in meat tenderization has been extensively studied (Huff-Lonergan *et al.* 2010). PEBP1 is a serine protease inhibitor also known as Raf kinase-inhibitory protein (Hengst *et al.* 2001), but may also be a calpain substrate (Chen *et al.* 2006).

CAPN1 was mostly positively, but also negatively, correlated in *Longissimus* and RA muscles with *L**, *a**, *b**, *C** and *h** (**Table 1** and **Figure 5**). CAPN1 degrades structural proteins and is therefore likely to influence light reflectance and scattering. The effect of CAPN1 on meat colour maybe influenced by HSPs, due to their protective action against structural proteins denaturation as described above (Gagaoua et al. 2017c). In another study, we found that the abundance of HSPA8 and of CAPN1 were positively correlated with each other as well as with the *L**, *a** and *b** colour coordinates (Gagaoua *et al.* 2015). One hypothesis is that inducible large HSP70 and μ-calpain interact in their influence on some meat colour parameters (Gagaoua *et al.* 2015).

The relationship between PEBP1 and colour of the *Longissimus* and SM muscles in 3 studies (Canto et al. 2015; Nair et al. 2016; Wu et al. 2016) appears to be independent of proteolysis, apoptosis, cell migration or signalling pathways , in agreement with the networks of proteins shown in **Figure 3A,B**. An increase in the abundance of PEBP1 has been reported in tough meat (Mahmood et al. 2018) and in colour-labile *Longissimus* steaks (Canto et al. 2015). In a previous study on pork *Longissimus* muscle, we found negative correlations between PEBP1 and *L** and *a** values (Kwasiborski et al. 2008). The role of PEBP1 in determining beef colour may also be related to its interactions with many multifunctional proteins and involvement in the coordination of cytoskeleton changes and energy metabolism (Schoentgen & Jonic 2020). This is also consistent with the observed relationship between PEPB1 and energy metabolism (**Figure 3A,B**). However,

the exact mechanism by which PEBP1 may affect meat colour is not clear and further investigations are needed, starting with validation of PEPB1 as a biomarker of beef colour.

8. Conclusion and future perspectives

It is a challenging task to improve the beef colour and colour stability during *post-mortem* storage and retail display. This integrative work reviewed the several biological pathways that are involved in meat colour development, including energy metabolism, heat shock proteins and oxidative stress, myofibril structure, signalling, proteolysis and apoptosis. The pathways underpinning meat colour are similar to those very recently described for beef tenderness, but with differences in the extent or impact of each pathway. Energy metabolism, particularly glycolytic and associated mechanisms seem to be, as expected, the major and predominant pathway impacting beef colour, and these pathways have previously been shown to influence not only beef colour, but also other quality traits such as tenderness, pH and water-holding capacity. The use of metabolomics as a novel tool has been proposed, to allow better targeting and identification of the potential biomarkers of the different energy metabolism pathways, which are related to colour enabling a deeper understanding of the underlying mechanisms. Identification of these biomarkers would provide future opportunities for *pre*- and *post-mortem* interventions that could improve the visual appearance and meat quality generated for the consumer, while addressing problematic meat colour issues for cattle producers and meat processors globally. Furthermore, this comprehensive review showed that by combining and comparing results of a number of proteomic studies (integromics) a high-throughput quantitative analysis of protein expression, modifications, and interactions can be achieved.

Apart from the role of energy metabolism in determining meat colour, the novelty in this research indicated that integromics can be used to identify the important role of the proteins associated with oxidative stress, cell redox and contraction, and particularly their interactions, in beef colour. The contractile and associated proteins associated with beef colour are proposed to have their influence through the changes to muscle structure which would influence light scattering from structural elements and the paleness of the meat surface. The oxidative/redox proteins are proposed to have a role in the onset of oxidation *post-mortem* hence influencing beef colour, and importantly, colour stability during storage and retail display.

Finally, the value of the information obtained in this review would serve as a one-stop-reference by proposing a comprehensive list of biomarkers that deserve particular attention in regards to muscle-to-meat conversion and the impact on beef colour. In the future, accurate quantification techniques of proteins such as selected reaction monitoring (SRM), multiple reaction monitoring

(MRM) or sequential window acquisition of all theoretical spectra (SWATH) could be used to validate the shortlisted top 27 biomarkers revealed by this integrative work.

Declaration of competing interest

The authors declared that there is no conflict of interest.

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Table 1. List of the 79 putative protein biomarkers by biological family reported in the 13 proteomic-based studies to be significantly correlated beef color traits.[1]

Protein biomarker names (*genes*)	UniProt Accession	Meat color traits	Muscles	Direction [2]	References
Chaperones & heat shock proteins					
Hsp20 (*HSPB6*)	O14558	Lightness (*L**)		-	Gagaoua et al. 2017a
		Yellowness (*b**)		-	Gagaoua et al. 2017a
		Chroma (*C**)		-	Gagaoua et al. 2017a
		Lightness (*L**)	L	-	Gagaoua et al. 2017c
		Chroma (*C**)		-	Gagaoua et al. 2017c
		Redness (*a**)		+	Gagaoua et al. 2018a
		Chroma (*C**)		+	Gagaoua et al. 2018a
Hsp27 (*HSPB1*)	P04792	Lightness (*L**)	L	-	Kim et al. 2008
		Redness (*a**)	L	-	Kim et al. 2008
		Redness (*a**)	L	-	Wu et al. 2016
		Redness (*a**)	L	-	Gagaoua et al. 2017a
		Redness (*a**)	L	-	Gagaoua et al. 2017c
		Yellowness (*b**)	L	-	Gagaoua et al. 2017c
		R630/580	L	+	Joseph et al. 2012
		Yellowness (*b**)	L	+	Gagaoua et al. 2017b
		Chroma (*C**)	L	+	Gagaoua et al. 2017b
		Lightness (*L**)	RA	+	Gagaoua et al. 2017a
αB-crystallin (*CRYAB*)	P02511	Lightness (*L**)		-	Gagaoua et al. 2015
		Redness (*a**)		-	Gagaoua et al. 2017b
		Lightness (*L**)	L	-	Gagaoua et al. 2017c
		Yellowness (*b**)		-	Gagaoua et al. 2017c
		Yellowness (*b**)		+	Gagaoua et al. 2015
		Chroma (*C**)		+	Gagaoua et al. 2015
Hsp40 (*DNAJA1)*	P31689	Lightness (*L**)		-	Gagaoua et al. 2017a
		Lightness (*L**)		-	Gagaoua et al. 2017b
		Lightness (*L**)		-	Gagaoua et al. 2017c
		Yellowness (*b**)		-	Gagaoua et al. 2017c
		Chroma (*C**)	L	-	Gagaoua et al. 2017c
		Hue angle (*h**)		-	Gagaoua et al. 2017c
		Redness (*a**)		+	Gagaoua et al. 2015
		Yellowness (*b**)		+	Gagaoua et al. 2015
		Chroma (*C**)		+	Gagaoua et al. 2015
Hsp60 (*HSPD1*)	P10809	Redness (*a**)	ST		Yu et al. 2017a
		Redness (*a**)	L	+	Wu et al. 2016
		MRA	L		Wu et al. 2016
Hsp70-8 (*HSPA8*)	P11142	Redness (*a**)	PM	-	Wu et al. 2016
		MRA	PM	-	Wu et al. 2016
		Lightness (*L**)	L	-	Gagaoua et al. 2015
		Redness (*a**)	L	-	Gagaoua et al. 2015
		Yellowness (*b**)	L	-	Gagaoua et al. 2017b
		Lightness (*L**)	L	-	Gagaoua et al. 2017b
		Redness (*a**)	L	-	Gagaoua et al. 2017b
		Chroma (*C**)	L	-	Gagaoua et al. 2017c
		Redness (*a**)	L	+	Gagaoua et al. 2017c
		Yellowness (*b**)	L	+	Gagaoua et al. 2015
		Chroma (*C**)	L	+	Gagaoua et al. 2015
Heat shock-related 70 kDa protein 2 Hsp72 (*HSPA2*)	P54652	Lightness (*L**)	L	-	Gagaoua et al. 2015
		Redness (*a**)	RA	-	Gagaoua et al. 2017a
		Yellowness (*b**)	RA	-	Gagaoua et al. 2017a
		Chroma (*C**)	RA	-	Gagaoua et al. 2017a
		Redness (*a**)	L	+	Gagaoua et al. 2017b
		Chroma (*C**)	L	+	Gagaoua et al. 2017b
		Redness (*a**)	L	+	Gagaoua et al. 2015
		Yellowness (*b**)	L	+	Gagaoua et al. 2015
		Chroma (*C**)	L	+	Gagaoua et al. 2015
		Hue angle (*h**)	L	+	Gagaoua et al. 2017a

Table 1. *Continued.*

Stress-70 protein, mitochondrial also Hsp70-Grp75 (*HSPA9*)	P38646	Lightness (*L**)	L	-	Gagaoua et al. 2015
		Yellowness (*b**)	L	-	Gagaoua et al. 2015
		Yellowness (*b**)	L	-	Gagaoua et al. 2017b
		Yellowness (*b**)	RA	+	Gagaoua et al. 2017a
		Redness (*a**)	L	+	Gagaoua et al. 2017a
		Yellowness (*b**)	L	+	Gagaoua et al. 2017a
		Chroma (*C**)	L	+	Gagaoua et al. 2017a
		Chroma (*C**)	L	+	Gagaoua et al. 2017c
Endoplasmic reticulum chaperone BiP (*HSPA5*)	P11021	MRA Redness (*a**)	PM	-	Wu et al. 2016
Stress-induced-phosphoprotein 1 (*STIP1*)	P31948	R630/580	L	+	Joseph et al. 2012
Catalytic, metabolism & ATP metabolic process					
A. Glycolysis and associated pathways					
Glycogen phosphorylase (*PYGM*)	P11217	Redness (*a**)	ST	-	Wu et al. 2015
		Redness (*a**)	L	+	Wu et al. 2016
		MRA	L	+	Wu et al. 2016
		Redness (*a**)	PM	-	Wu et al. 2016
		MRA	PM	-	Wu et al. 2016
Phosphoglucomutase 1 (*PGM1*)	P36871	Redness (*a**)	ST	-	Yu et al. 2017a
		Redness (*a**)	L	+	Canto et al. 2015
		R630/580	L	+	Canto et al. 2015
		Lightness (*L**)	L	+	Gagaoua et al. 2017a
		Hue angle (*h**)	L	+	Gagaoua et al. 2017a
		Yellowness (*b**)	RA	+	Gagaoua et al. 2017a
		Redness (*a**)	L	-	Gagaoua et al. 2017c
		Hue angle (*h**)	L	+	Gagaoua et al. 2017c
		Redness (*a**)	PM	-	Wu et al. 2016
		MRA	PM	-	Wu et al. 2016
Fructose-bisphosphate aldolase A (*ALDOA*)	P04075	Redness (*a**)	ST	-	Wu et al. 2015
		MRA	ST		Wu et al. 2015
		Redness (*a**)	L		Wu et al. 2016
		MRA	SM		Nair et al. 2016
Triosephosphate isomerase 1 (*TPI1*)	P60174	Redness (*a**)	ST	-	Wu et al. 2015
		MRA	ST	-	Wu et al. 2015
		Redness (*a**)	L	-	Wu et al. 2016
		MRA	L	-	Wu et al. 2016
		Redness (*a**)	PM	-	Wu et al. 2016
		MRA	SM	+	Nair et al. 2016
		R630/580	SM	+	Nair et al. 2016
		Yellowness (*b**)	L	+	Gagaoua et al. 2018a
Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)	P04406	Redness (*a**)		+	Canto et al. 2015
		R630/580	L	+	Canto et al. 2015
		Redness (*a**)		-	Wu et al. 2016
Phosphoglycerate kinase 1 (*PGK1*)	P00558	MRA	L	+	Wu et al. 2016
Phosphoglycerate mutase 2 (*PGAM2*)	P15259	MRA R630/580	SM	-	Nair et al. 2016
Enolase 3 (*ENO3*)	P13929	Redness (*a**)	L	+	Joseph *et al.* 2012
		Redness (*a**)	ST	-	Yu et al. 2017a
		MRA	SM	-	Nair et al. 2016
		R630/580	SM	-	Nair et al. 2016
		Lightness (*L**)	L	+	Gagaoua et al. 2015
		Yellowness (*b**)	L	-	Gagaoua et al. 2015
		Chroma (*C**)	L	-	Gagaoua, 2015
		Hue angle (*h**)	L	+	Gagaoua et al. 2017a
		Chroma (*C**)	RA	+	Gagaoua et al. 2017a
		Lightness (*L**)	L	-	Gagaoua et al. 2017b
		Yellowness (*b**)	L	+	Gagaoua et al. 2017c
		MRA	L	+	Wu et al. 2016
		Redness (*a**)	PM	+	Wu et al. 2016
		MRA	PM	+	Wu et al. 2016
		Redness (*a**)	PM	-	Wu et al. 2016

Table 1. *Continued.*

Enolase 1 (*ENO1*)	P06733	Lightness (*L**)	L	-	Gagaoua et al. 2018a
Enolase 2 (*ENO2*)	P09104	Redness (*a**)	ST	-	Yu et al. 2017
Pyruvate kinase M 2 (*PKM2*)	P14618	Redness (*a**)	ST	-	Wu et al. 2015
		MRA	ST	-	Wu et al. 2015
		Redness (*a**)	L	-	Wu et al. 2016
		MRA	L	-	Wu et al. 2016
		Redness (*a**)	PM	-	Wu et al. 2016
		MRA	PM	-	Wu et al. 2016
		Redness (*a**)	L	+	Canto et al. 2015
		R630/580	L	+	Canto et al. 2015
Lactate dehydrogenase (*LDH*)	P00338	Redness (*a**)	ST	+	Wu et al. 2015
		MRA	ST	+	Wu et al. 2015
		Lightness (*L**)	RA	+	Gagaoua et al. 2017a
		Redness (*a**)	L	+	Gagaoua et al. 2017b
		Lightness (*L**)	L	-	Gagaoua et al. 2017c
Pyruvate dehydrogenase (*PDHA1*)	P08559	Redness (*a**)	L	+	Joseph et al. 2012
		R630/580			
Pyruvate dehydrogenase protein X component (*PDHX*)	O00330	Redness (*a**)	PM	+	Wu et al. 2016
Glycerol-3-phosphate dehydrogenase [NAD(+)] (*GPD1*)	P21695	Redness (*a**)	L	+	Wu et al. 2016
		MRA			
B. Tricarboxylic acid cycle and associated pathways					
Malate dehydrogenase 1 (*MDH1*)	P40925	MRA	ST	-	Wu et al. 2015
		MRA	L	-	Wu et al. 2016
		Redness (*a**)	L	-	Wu et al. 2016
		Redness (*a**)	L	+	Gagaoua et al. 2015
		Chroma (*C**)	L	+	Gagaoua, 2015
		Lightness (*L**)	L	+	Gagaoua et al. 2018a
		Yellowness (*b**)	L	-	Gagaoua et al. 2018a
Aconitate hydratase, mitochondrial (*ACO2*)	Q99798	Redness (*a**)	PM	-	Joseph et al. 2012
3-hydroxyisobutyrate dehydrogenase, mitochondrial (*HIBADH*)	P31937	Chroma (*C**)	L	+	Yang et al. 2018
		OCR			
Glutamate dehydrogenase 1, mitochondrial (*GLUD1*)	P00367	MRA	L	-	Yang et al. 2018
Isocitrate dehydrogenase (*IDH1*)	O75874	Lightness (*L**)	RA	-	Gagaoua et al. 2017a
		Redness (*a**)	L	+	
		Chroma (*C**)	L	+	
		Redness (*a**)	RA	+	
		Chroma (*C**)	RA	-	
Succinyl-CoA:3-ketoacid-coenzyme A transferase (*OXCT1*)	P55809	Redness (*a**)	ST	+	Yu et al. 2017a
ATP synthase F(0) complex subunit B1, mitochondrial (*ATP5F1*)	P24539	Redness (*a**)	ST		Yu et al. 2017a
		Redness (*a**)	L	+	Wu et al. 2016
		MRA	L		Wu et al. 2016
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6 (*NDUFA6*)	P56556	Redness (*a**)	ST	+	Yu et al. 2017a
NADH dehydrogenase [ubiquinone] 1 subunit C2 (*NDUFC2*)	E9PQ53	Redness (*a**)	ST	+	Yu et al. 2017a
Cytochrome c oxidase subunit NDUFA4 (*NDUFA4*)	O00483	Redness (*a**)	ST	+	Yu et al. 2017a
C. Other catalytic and ATP metabolic pathways grouping oxidoreductase, transferase, hydrolase, lyase & kinase					
Creatine kinase M type (*CKM*)	P06732	Redness (*a**)	L		Joseph et al. 2012
		MRA	SM	+	Nair et al. 2016
		R630/580	SM		Nair et al. 2016
		MRA	L		Yang et al. 2018
Retinal dehydrogenase 1 (*ALDH1A1*)	P00352	Redness (a*)	L	+	Wu et al. 2016
		MRA		+	Wu et al. 2016
		Redness (a*)		+	Gagaoua et al. 2018a
		Hue angle (*h**)		-	Gagaoua et al. 2018a

Table 1. *Continued.*

Aldose reductase (*ALDR1*)	P15121	Redness (*a**)	L	+	Joseph et al. 2012
Cytosol aminopeptidase 3 (*LAP3*)	P28838	Redness (*a**) MRA	ST	+	Wu et al. 2015
Adenylate kinase isoenzyme 1 (*AK1*)	P00568	Redness (*a**) MRA	L	+	Wu et al. 2016
Protein-L-isoaspartate O-methyltransferase (*PCMT1*)	P22061	Redness (*a**)	ST	-	Yu et al. 2017a
Flavin reductase (NADPH) (*BLVRB*)	P30043	Redness (*a**)	ST	+	Yu et al. 2017a
Mitochondrial peptide methionine sulfoxide reductase (*MSRA*)	Q9UJ68	Redness (*a**) MRA R630/580 MRA	L	+	Wu et al. 2016 Wu et al. 2016 Joseph et al. 2012 Joseph et al. 2012
Adenosylhomocysteinase (*AHCY*)	P23526	Redness (*a**) MRA Redness (*a**) MRA	L L PM PM	+ + - -	Wu et al. 2016
Lactoylglutathione lyase (*GLO1*)	Q04760	Redness (*a**) MRA	L	+	Wu et al. 2016
Oxidative stress & cell redox homeostasis					
DJ-1 (*PARK7*)	Q99497	Redness (*a**) MRA Redness (*a**) Chroma (*C**) Redness (*a**)	ST ST L L L	 - 	Wu et al. 2015 Wu et al. 2015 Gagaoua et al. 2017b Gagaoua et al. 2017b Yang et al. 2018
Glutathione peroxidase 1 (*GPX1*)	P07203	Redness (*a**)	ST	-	Yu et al. 2017a
Glutathione S-transferase P (*GSTP1*)	P09211	Redness (*a**) MRA	PM	-	Wu et al. 2016
Peroxiredoxin 1 (*PRDX1*)	Q06830	Redness (*a**) MRA	L	+	Wu et al. 2016
Peroxiredoxin 2 (*PRDX2*)	P32119	R630/580 Redness (*a**) MRA Chroma (*C**) OCR Redness (*a**) MRA	L L L L L PM PM	+ + + + + - -	Joseph et al. 2012 Wu et al. 2016 Wu et al. 2016 Yang et al. 2018 Yang et al. 2018 Wu et al. 2016 Wu et al. 2016
Thioredoxin-dependent peroxide reductase (*PRDX3*)	P30048	Redness (*a**) MRA MRA	L L PM	+ + -	Yang et al. 2018 Yang et al. 2018 Wu et al. 2016
Peroxiredoxin 6 (*PRDX6*)	P30041	Redness (*a**) MRA Redness (*a**) Yellowness (*b**) Hue angle (*h**) Redness (*a**) Yellowness (*b**) Chroma (*C**) Lightness (*L**) OCR Redness (*a**) Chroma (*C**) Redness (*a**) Chroma (*C**) Hue angle (*h**) MRA	ST ST L RA RA RA L L L L L L L L L PM	- - + + + + - - - + + + + + - -	Wu et al. 2015 Wu et al. 2015 Gagaoua et al. 2015 Gagaoua et al. 2017a Gagaoua et al. 2017a Gagaoua et al. 2017a Gagaoua et al. 2017b Gagaoua et al. 2017b Gagaoua et al. 2017b Yang et al. 2018 Yang et al. 2018 Yang et al. 2018 Gagaoua et al. 2017c Gagaoua et al. 2017c Gagaoua et al. 2017c Wu et al. 2016

Table 1. *Continued.*

Superoxide Dismutase (*SOD1*)	P00441	Redness (*a**)	L	-	Gagaoua et al. 2015
		Yellowness (*b**)	L	-	Gagaoua et al. 2017b
		Redness (*a**)	L	+	Gagaoua et al. 2018a
		Yellowness (*b**)	L	+	Gagaoua et al. 2018a
		Chroma (*C**)	L	+	Gagaoua et al. 2018a
		Redness (*a**)	L	-	Gagaoua et al. 2017c
		Redness (*a**)	PM	+	Wu et al. 2016
		MRA	PM	+	Wu et al. 2016
Protein disulfide-isomerase (*P4HB*)	P07237	Redness (*a**)	PM	-	Wu et al. 2016
Contractile & associated proteins					
Myosin light chain 1 (*MLC1*)	Q15049	Redness (*a**)	L	+	Canto et al. 2015
		Yellowness (*b**)			Gagaoua et al. 2017b
		Yellowness (*b**)			Gagaoua et al. 2017c
		Chroma (*C**)			Gagaoua et al. 2017c
Myosin regulatory light chain 2, (*MYL2*)	P10916	Redness (*a**)	L	+	Canto et al. 2015
		R630/580			
Myosin-7 (*MYH7*)	P12883	Redness (*a**)	ST	-	Yu et al. 2017a
		Lightness (*L**)	L	-	Gagaoua et al. 2015
		Yellowness (*b**)	L	+	Gagaoua et al. 2015
		Chroma (*C**)	L	+	Gagaoua et al. 2015
		Lightness (*L**)	RA	-	Gagaoua et al. 2017a
		Yellowness (*b**)	L	+	Gagaoua et al. 2017a
		Lightness (*L**)	L	-	Gagaoua et al. 2017b
Myosin-2 (*MYH2*)	Q9UKX2	Lightness (*L**)	L	+	Gagaoua et al. 2015
		Hue angle (*h**)	RA	-	Gagaoua et al. 2017a
		Chroma (*C**)	L	-	Gagaoua et al. 2017a
		Chroma (*C**)	RA	+	Gagaoua et al. 2017a
		Lightness (*L**)	L	-	Gagaoua et al. 2017b
		Yellowness (*b**)	L	-	Gagaoua et al. 2017b
		Chroma (*C**)	L	-	Gagaoua et al. 2017b
		Redness (*a**)	L	+	Gagaoua et al. 2017c
		Yellowness (*b**)	L	+	Gagaoua et al. 2017c
		Chroma (*C**)	L	+	Gagaoua et al. 2017c
		Hue angle (*h**)	L	-	Gagaoua et al. 2017c
Myosin-1 (*MYH1*)	P12882	Lightness (*L**)	RA	+	Gagaoua et al. 2017a
		Yellowness (*b**)	L	+	Gagaoua et al. 2017b
		Chroma (*C**)	L	+	Gagaoua et al. 2017b
		Redness (*a**)	L	-	Gagaoua et al. 2018a
		Yellowness (*b**)	L	-	Gagaoua et al. 2018a
		Chroma (*C**)	L	-	Gagaoua et al. 2018a
F-actin-capping protein subunit beta (*CAPZB*)	P47756	Redness (*a**)	L	-	Gagaoua et al. 2017b
		Chroma (*C**)			Gagaoua et al. 2017b
Actin, alpha skeletal muscle (*ACTA1*)	P68133	Lightness (*L**)	L	-	Gagaoua et al. 2015
		Lightness (*L**)	RA	-	Gagaoua et al. 2017a
		Hue angle (*h**)	RA	+	Gagaoua et al. 2017a
		Lightness (*L**)	L	-	Gagaoua et al. 2018a
		Redness (*a**)	L	-	Gagaoua et al. 2018a
		Yellowness (*b**)	L	-	Gagaoua et al. 2018a
		Chroma (*C**)	L	-	Gagaoua et al. 2018a
		Hue angle (*h**)	L	-	Gagaoua et al. 2018a
Alpha-actinin-3 (*ACTN3*)	Q0III9	Yellowness (*b**)	L	-	Gagaoua et al. 2018a
Titin (*TTN*)	Q8WZ42	Lightness (*L**)	L	-	Gagaoua et al. 2018a
		Redness (*a**)		+	
		Yellowness (*b**)		+	
		Chroma (*C**)		+	
Myosin binding protein-H (*MYBPH*)	Q13203	Lightness (*L**)	L	+	Gagaoua et al. 2015
		Yellowness (*b**)	L	-	Gagaoua et al. 2015
		Chroma (*C**)	L	-	Gagaoua et al. 2015
		Hue angle (*h**)	L	-	Gagaoua et al. 2017a
		Lightness (*L**)	RA	-	Gagaoua et al. 2017a
		Hue angle (*h**)	L	-	Gagaoua et al. 2017c

Table 1. *Continued.*

Proteolysis & associated proteins					
Calpain-1 catalytic subunit (*CAPN1*)	P07384	Lightness (*L**)	L	-	Gagaoua et al. 2015
		Yellowness (*b**)	L	+	Gagaoua et al. 2015
		Chroma (*C**)	L	+	Gagaoua et al. 2015
		Redness (*a**)	RA	+	Gagaoua et al. 2017a
		Chroma (*C**)	RA	+	Gagaoua et al. 2017a
		Yellowness (*b**)	L	+	Gagaoua et al. 2017b
		Yellowness (*b**)	L	-	Gagaoua et al. 2018a
		Hue angle (*h**)	L	-	Gagaoua et al. 2018a
		Lightness (*L**)	L	+	Gagaoua et al. 2017c
		Chroma (*C**)	L	+	Gagaoua et al. 2017c
Calpain-2 catalytic subunit (*CAPN2*)	P17655	Lightness (*L**) Chroma (*C**)	L	+	Gagaoua et al. 2017c
Proteasome subunit beta type-2 (*PSMB2*)	P49721	Redness (*a**)	ST	-	Yu et al. 2017a
Binding, cofactor & transport proteins, signaling or apoptosis					
Serum albumin (*ALB*)	P02768	Redness (*a**)	ST	-	Wu et al. 2015
		MRA	ST		Wu et al. 2015
		Redness (*a**)	PM		Wu et al. 2016
		MRA	PM		Wu et al. 2016
Hemoglobin subunit beta (*HBB*)	P68871	Redness (*a**)	ST	+	Yu et al. 2017a
Tripartite motif-containing protein 72 (*TRIM72*)	Q6ZMU5	Lightness (*L**)	L	-	Gagaoua et al. 2018a
Four and a half LIM domains 1 (*FHL1*)	Q13642	Lightness (*L**)	L	-	Gagaoua et al. 2018a
		Redness (*a**)		+	
		Chroma (*C**)		+	
Myoglobin (*MB*)	P02144	Redness (*a**)	L	-	Wu et al. 2016
		MRA	L	-	
		Redness (*a**)	PM	+	
		MRA	PM	+	
Fibrinogen beta chain (*FGB*)	P02675	Redness (*a**)	ST	+	Yu et al. 2017a
Phosphatidylethanolamine-binding protein 1 (*PEBP1*)	P30086	Redness (*a**)	L	-	Canto et al. 2015
		R630/580	L	-	Canto et al. 2015
		Redness (*a**)	L	+	Wu et al. 2016
		MRA	SM	-	Nair et al. 2016
Annexin A5 (*ANXA5*)	P08758	MRA	ST	-	Wu et al. 2015
		Redness (*a**)	PM		Wu et al. 2016
Cellular tumor antigen p53 (*TP53*)	P04637	Lightness (*L**)	L	+	Gagaoua et al. 2017b
		Hue angle (*h**)			Gagaoua et al. 2017b
		Yellowness (*b**)			Gagaoua et al. 2017c
Polyubiquitin-B (*UBB*)	P0CG47	Redness (*a**)	L	+	Wu et al. 2016
		MRA			
Histone H2AX (*H2AFX*)	P16104	Yellowness (*b**)	L	+	Gagaoua et al. 2017c
		Hue angle (*h**)			
Fatty acid-binding protein (*FABP3*)	P05413	MRA	L	+	Wu et al. 2016

[1] Papers that reported protein abundances only or comparisons between ageing times of meat and effect on meat color (9 studies) were not included in this list, but those proteins were cited in the manuscript.

[2] (+) positively related; (-) negatively related.

Abbreviations: L: *Longissimus* muscle (the term *Longissimus* signifies in this review m. *longissimus dorsi*, m. *longissimus lumborum* and *m. longissimus thoracis*); RA: *Rectus abdominis*; ST: *Semitendinosus;* SM: *Semimembranosus;* PM: *Psoas major;* MRA: Metmyoglobin reducing activity; OCR: Oxygen consumption rate; R630/580: a reflectance ratio indicating the oxymyoglobin to metmyoglobin levels.

Figure captions

Figure 1. Venn diagram summarizing the distribution of the 79 putative markers among the 5 muscles: L: *Longissimus* muscle*;* RA: *Rectus abdominis*; ST: *Semitendinosus;* SM: *Semimembranosus* and PM: *Psoas major*. The total number of proteins for each muscle is given in white circles near each muscle abbreviation name. The number of proteins specific to muscle or common among muscle is given in black bold type characters. The corresponding gene names of the proteins are given for each situation and full details of the proteins and their UniprotIDs are given in **Table 1**.

Figure 2. Venn diagrams summarizing **A)** 73 proteins from the 79 putative markers across all breed/animal types and muscles; and **B)** 54 proteins from 59 putative markers of *Longissimus* muscle identified to be correlated with *L*, a*, b** beef colour coordinates. For the full protein names and UniprotIDs refer to **Table 1**. The total number of proteins for each colour coordinate is given in white circles near each colour parameter. The numbers of proteins specific to each colour trait or common among the colour traits are given in black bold type characters. Only 6 proteins (STIP1, PGAM2, PGK1, HIBADH, GLUD1 and FABP3) were not yet identified to be correlated to *L*a*b** colour coordinates in the whole metadata. The green and red proteins in bold character represent positive and negative relationships identified irrespective of the identified factors from a minimum of two studies (**Table 1** and **Figure S3**). The common proteins to the three colour traits in A and B are given at the right of the Venn diagrams.

Figure 3. STRING functional interaction networks. **A)** Protein-protein network linking the proteins (n = 79) identified by proteomic studies to be related to beef colour traits from the five muscles (**Table 1**). The interaction map was generated from a web-based search of the STRING 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. The secreted proteins (n = 8) as revealed by ProteINSIDE tool (http://www.proteinside.org/) are shown by ovals in black (n = 3, proteins secreted by classical pathways) and red (n = 5, proteins secreted by non-classical pathways) for each protein. **B)** Protein-protein network linking the *Longissimus* proteins (n = 59) identified by proteomic studies to be related to beef colour traits (**Table 1**). The secreted proteins (n = 4) as revealed by ProteINSIDE tool (http://www.proteinside.org/) are shown by ovals red (proteins secreted by non-classical pathways) for each protein. Considering the limitation of the GO annotation of genes in bovine, we converted their UniprotIDs to orthologous human EntrezGene IDs using BioMart (http://www.ensembl.org/biomart/). Colour code symbols and description of the 79 proteins identified in STRING database are given in **Table S3**.

Figure 4. Simplified metabolic role and localisation of the 35 proteins belonging to catalytic, metabolism & ATP metabolic process that is the largest pathway of putative biomarkers identified in this integromics study. The proteins were categorised into three main sub-pathways that are **A)** Glycolysis and associated pathways (surrounded proteins in blue): PYGM, PGM1, ALDOA, TPI1, GAPDH, PGK1, PGAM2, ENO1, ENO2, ENO3, PKM2, LDH, PDHA1, PDHX, GPD1 (n = 15); **B)** Tricarboxylic acid cycle and associated pathways (surrounded proteins in green): MDH1, ACO2, HIBADH, GLUD1, IDH1, OXCT1, ATP5F1, NDUFA6, NDUFC2, NDUFA4 (n = 10); and **C)** Other catalytic and ATP metabolic pathways grouping oxidoreductase, transferase, hydrolase, lyase & kinase (surrounded proteins in orange): CKM, ALDH1A1, ALDR1, LAP3, AK1, PCMT1, BLVRB, MSRA, AHCY, GLO1 (n = 10). For the full protein names and UniprotIDs refer to **Table 1**.

Figure 5. A summary by biological family and muscle or by CIE-colour coordinate of the direction of the correlations between the putative markers and beef colour traits. **A)** Correlations by muscle within the 79 proteins whatever the colour trait. **B)** Correlations by colour coordinate (*L*a*b**) within the 54 proteins of *Longissimus* muscle alone (the muscle that have the high number of proteins and considered as a reference in the literature). The negative correlations are highlighted in red, the positive in blue and those that are both negative and positive are highlighted in yellow colour. For more details of these latter correlations (both positive and negative) refer to **Table 1**. The 3 sub-pathways of the "catalytic, metabolism & ATP metabolic process" are separated by roman numerals; **I**: Glycolysis and associated pathways; **II:** Tricarboxylic acid cycle and associated pathways; **III:** Other catalytic and ATP metabolic pathways grouping oxidoreductase, transferase, hydrolase, lyase & kinase.

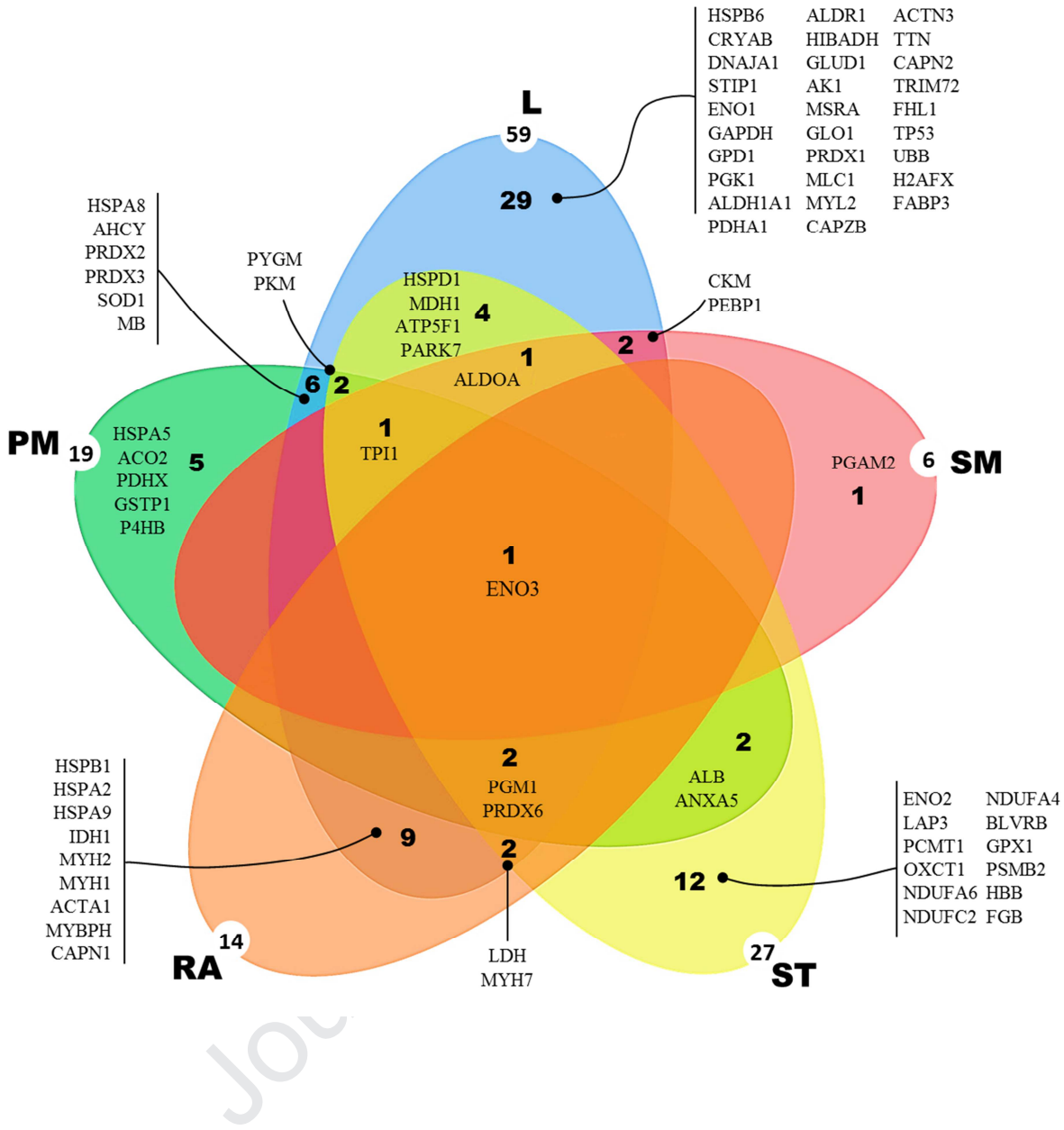
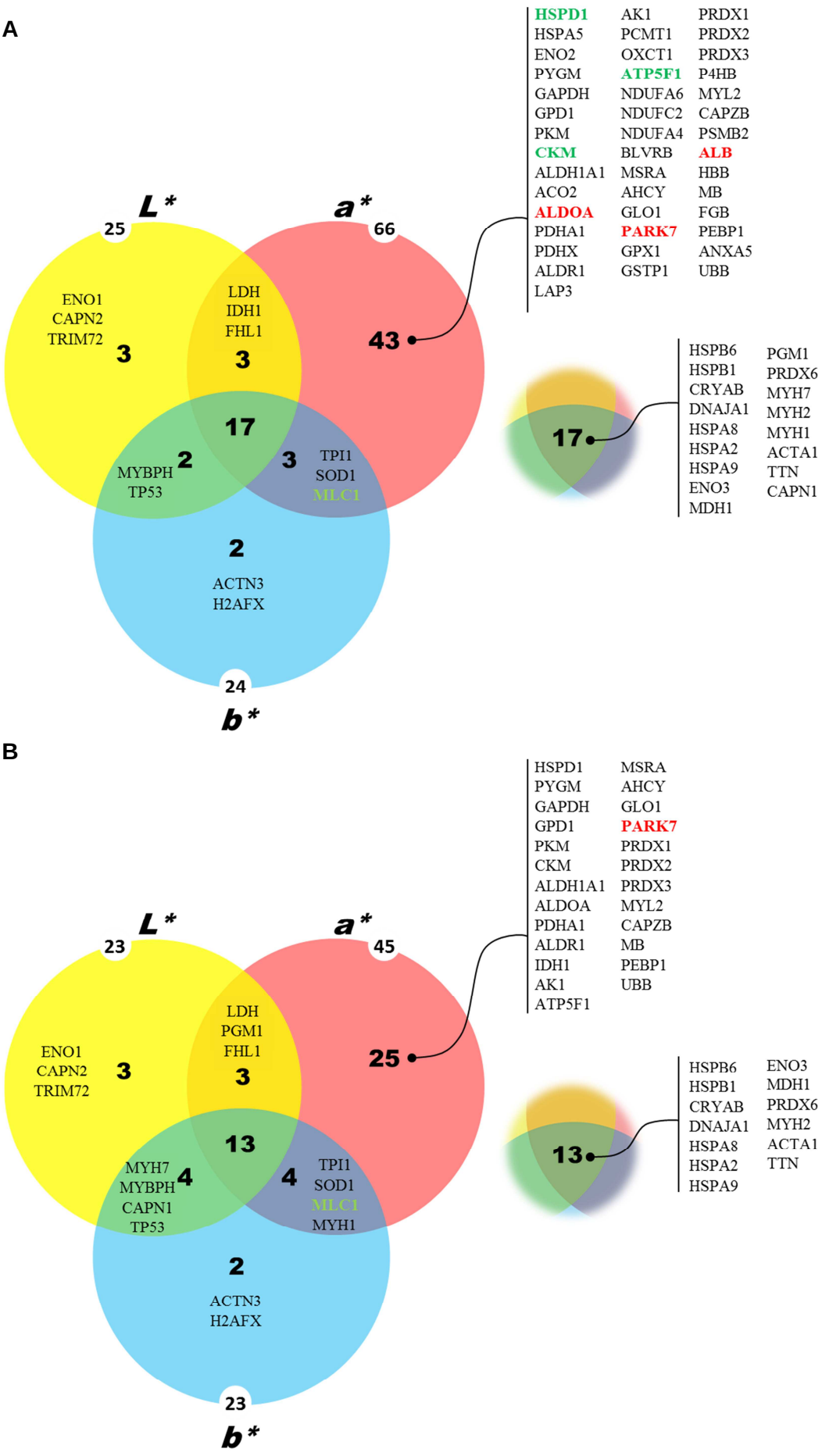


Figure 1.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L*
25
a*
66
b*
24
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CAPN2
TRIM72
3
LDH
IDH1
FHL1
3
17
MYBPH
TP53
2
3
TPI1
SOD1
MLC1
2
ACTN3
H2AFX
43
HSPD1
HSPA5
ENO2
PYGM
GAPDH
GPD1
PKM
CKM
ALDH1A1
ACO2
ALDOA
PDHA1
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ALDR1
LAP3
AK1
PCMT1
OXCT1
ATP5F1
NDUFA6
NDUFC2
NDUFA4
BLVRB
MSRA
AHCY
GLO1
PARK7
GPX1
GSTP1
PRDX1
PRDX2
PRDX3
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PGM1
PRDX6
MYH7
MYH2
MYH1
ACTA1
TTN
CAPN1
B
L*
23
a*
45
b*
23
ENO1
CAPN2
TRIM72
3
LDH
PGM1
FHL1
3
13
MYH7
MYBPH
CAPN1
TP53
4
4
TPI1
SOD1
MLC1
MYH1
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ACTN3
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PDHA1
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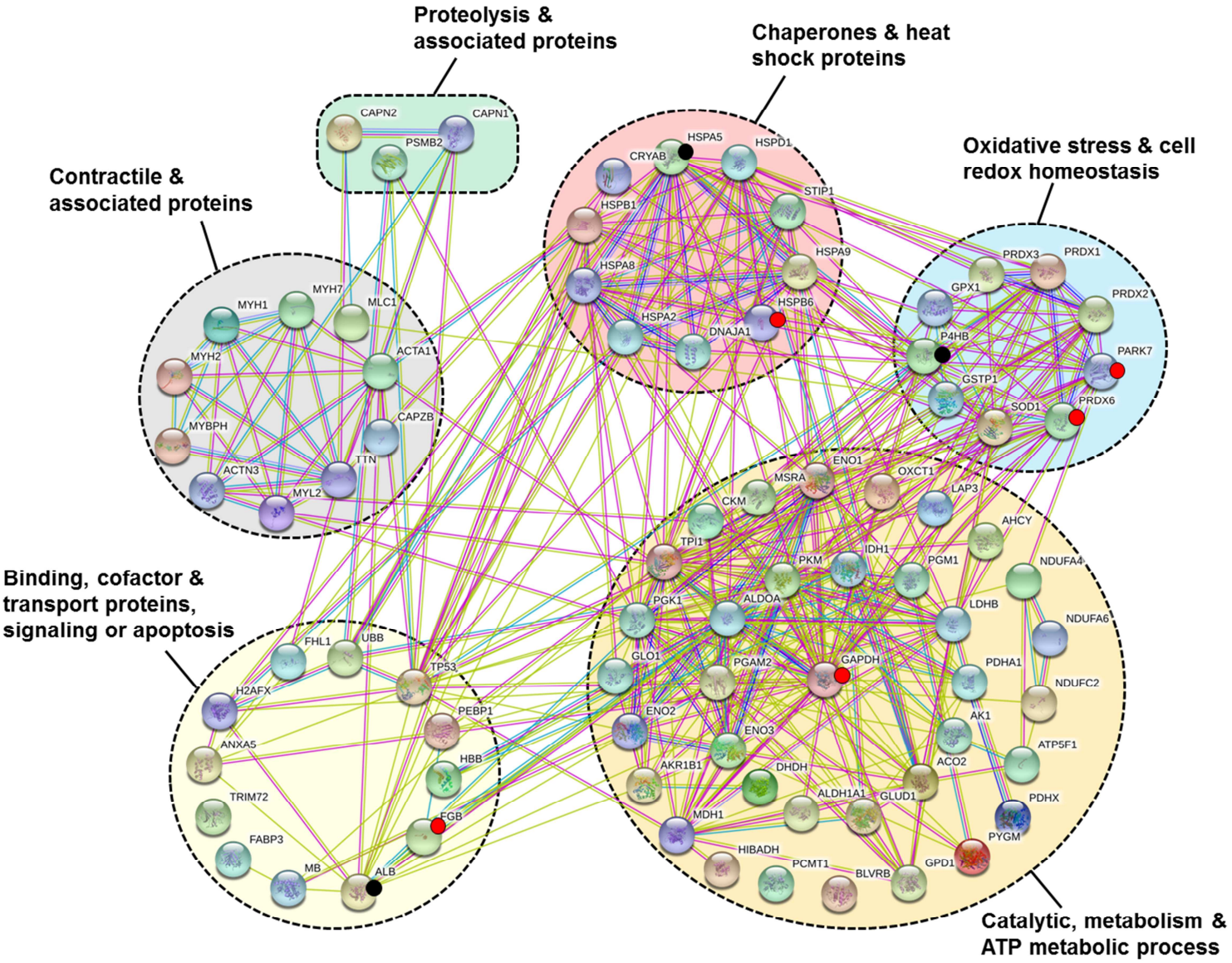
ACTA1
TTN

Figure 2.


Proteolysis &
associated proteins
Chaperones & heat
shock proteins
Oxidative stress & cell
redox homeostasis
Contractile &
associated proteins
Binding, cofactor &
transport proteins,
signaling or apoptosis
Catalytic, metabolism &
ATP metabolic process
CAPN2
CAPN1
PSMB2
HSPA5
HSPD1
CRYAB
HSPB1
STIP1
HSPA8
HSPA9
HSPA2
HSPB6
DNAJA1
PRDX3
PRDX1
GPX1
PRDX2
P4HB
PARK7
GSTP1
SOD1
PRDX6
MYH7
MYH1
MLC1
ACTA1
MYH2
CAPZB
MYBPH
TTN
ACTN3
MYL2
ENO1
MSRA
OXCT1
LAP3
CKM
AHCY
TPI1
IDH1
PKM
PGM1
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ALDOA
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NDUFA6
GLO1
PGAM2
GAPDH
PDHA1
NDUFC2
ENO2
AK1
ENO3
ATP5F1
AKR1B1
DHDH
ACO2
ALDH1A1
GLUD1
PDHX
MDH1
PYGM
HIBADH
PCMT1
BLVRB
GPD1
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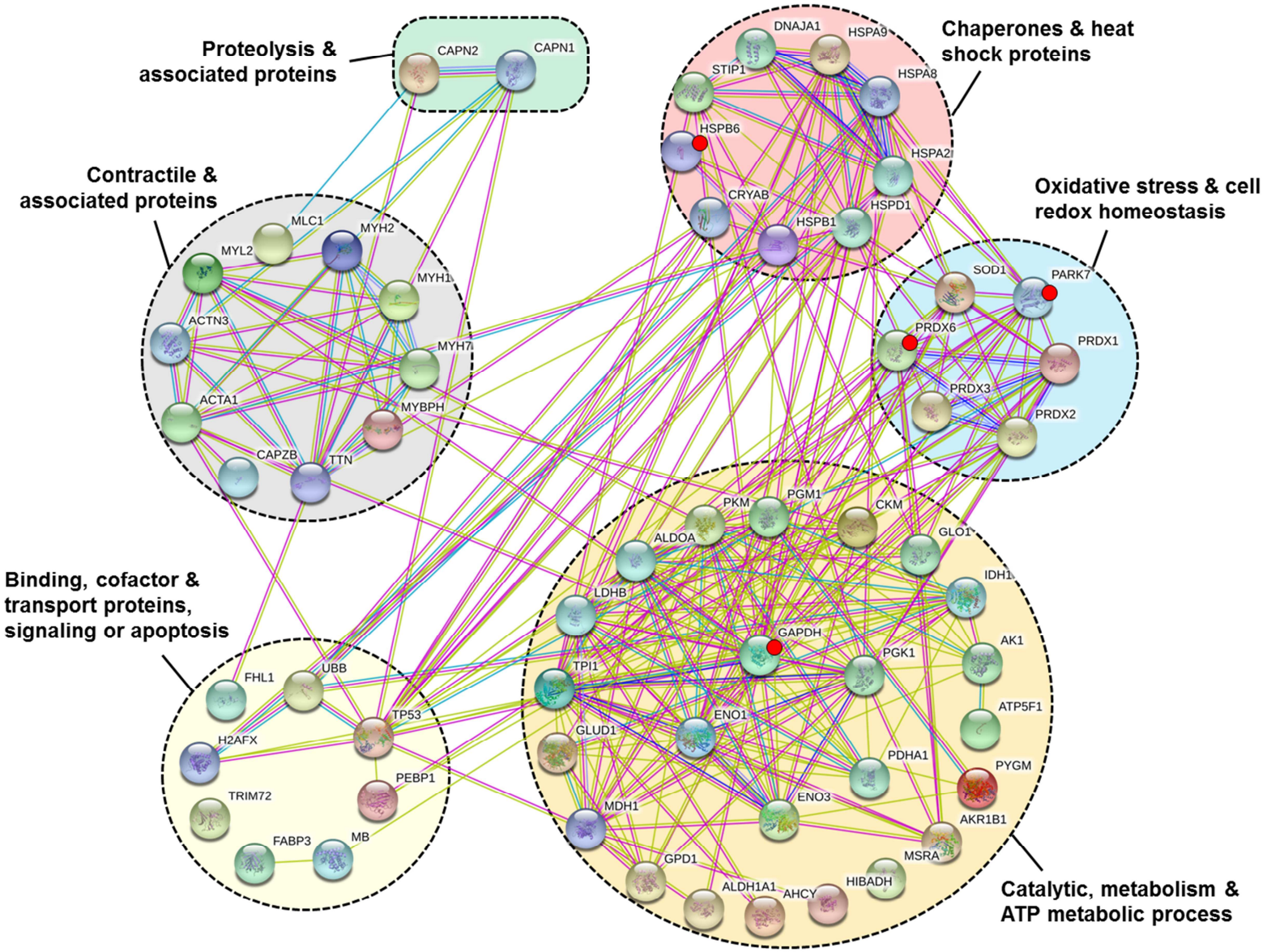
ANXA5
HBB
TRIM72
FGB
FABP3
MB
ALB

Figure 3A.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CAPN1
Chaperones & heat shock proteins
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CRYAB
HSPD1
HSPB1
Contractile & associated proteins
MLC1
MYH2
MYL2
MYH1
ACTN3
MYH7
ACTA1
MYBPH
CAPZB
TTN
Oxidative stress & cell redox homeostasis
SOD1
PARK7
PRDX6
PRDX1
PRDX3
PRDX2
Binding, cofactor & transport proteins, signaling or apoptosis
FHL1
UBB
TP53
H2AFX
PEBP1
TRIM72
FABP3
MB
PKM
PGM1
CKM
ALDOA
GLO1
IDH1
LDHB
GAPDH
AK1
PGK1
TPI1
ATP5F1
ENO1
GLUD1
PDHA1
PYGM
ENO3
MDH1
AKR1B1
MSRA
GPD1
HIBADH
ALDH1A1
AHCY
Catalytic, metabolism & ATP metabolic process

Figure 3B.

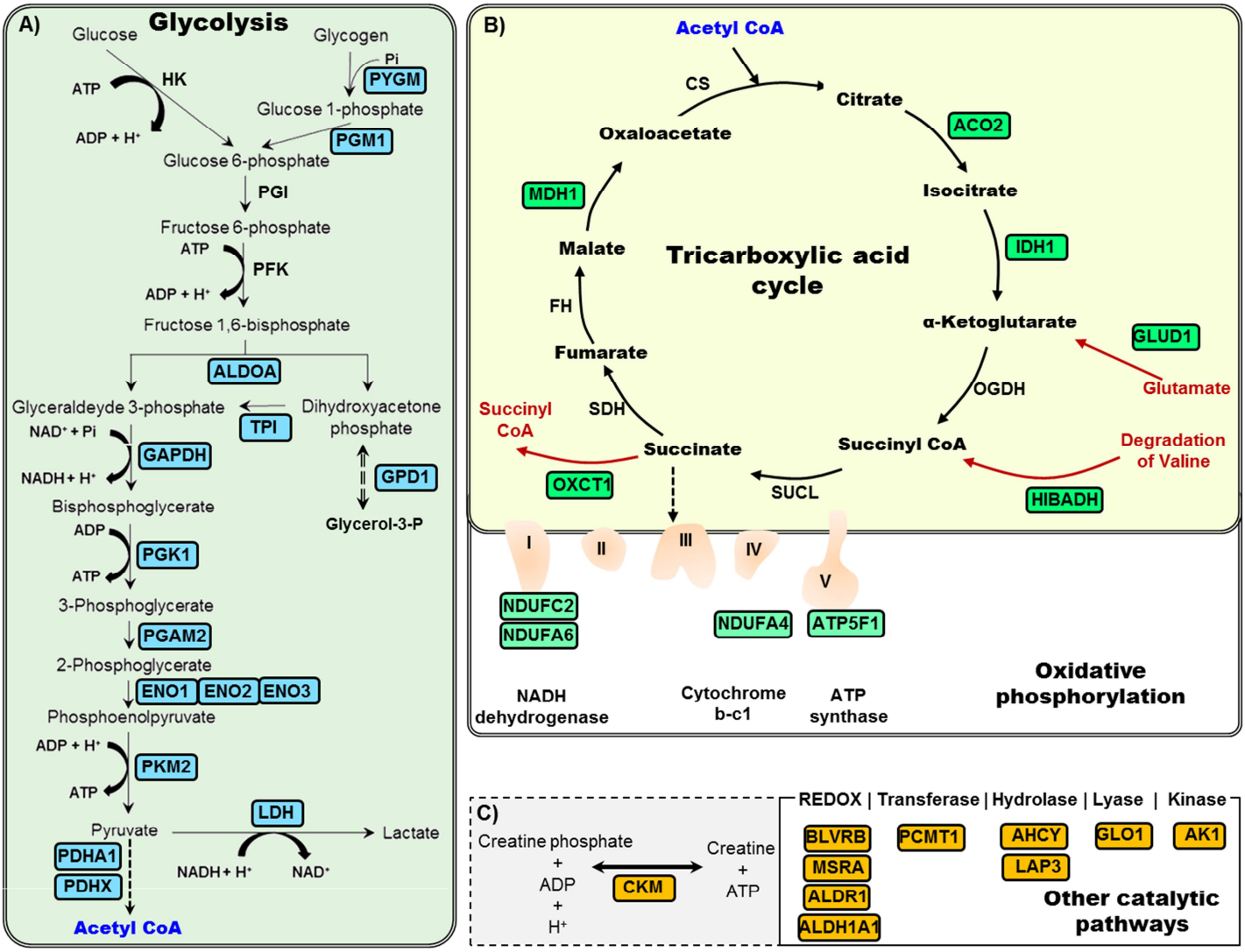

Figure 4.

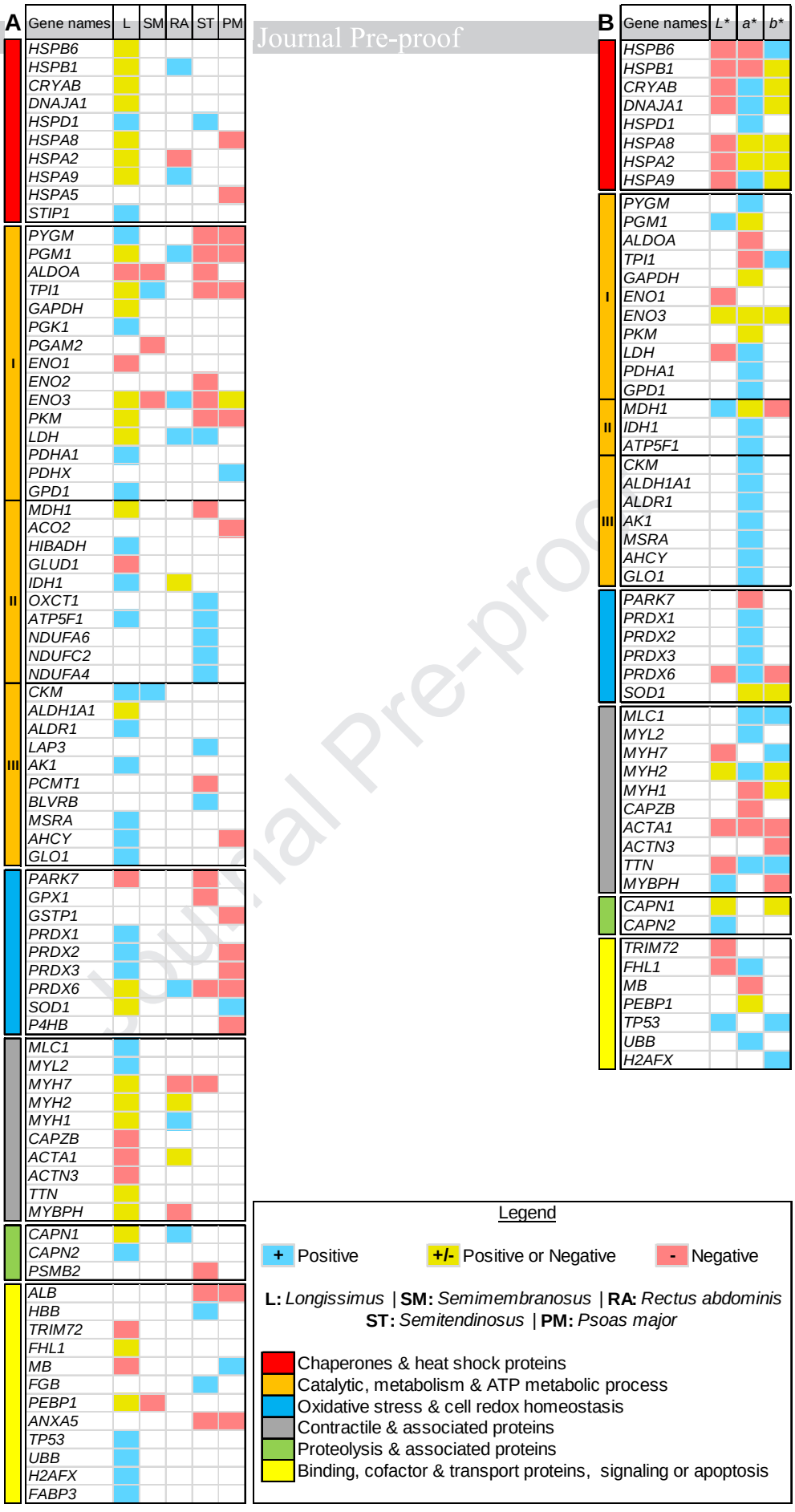

Figure 5.

Highlights

- Proteomics is a rapidly growing area of muscle foods characterization
- Biomarkers of beef colour are reviewed and categorized into 6 biological pathways
- 79 putative biomarkers of beef colour were identified from 5 different muscles
- 27 putative biomarkers of beef colour were proposed for validation
- β-enloase (ENO3) is a generic biomarker irrespective of muscle type and colour trait