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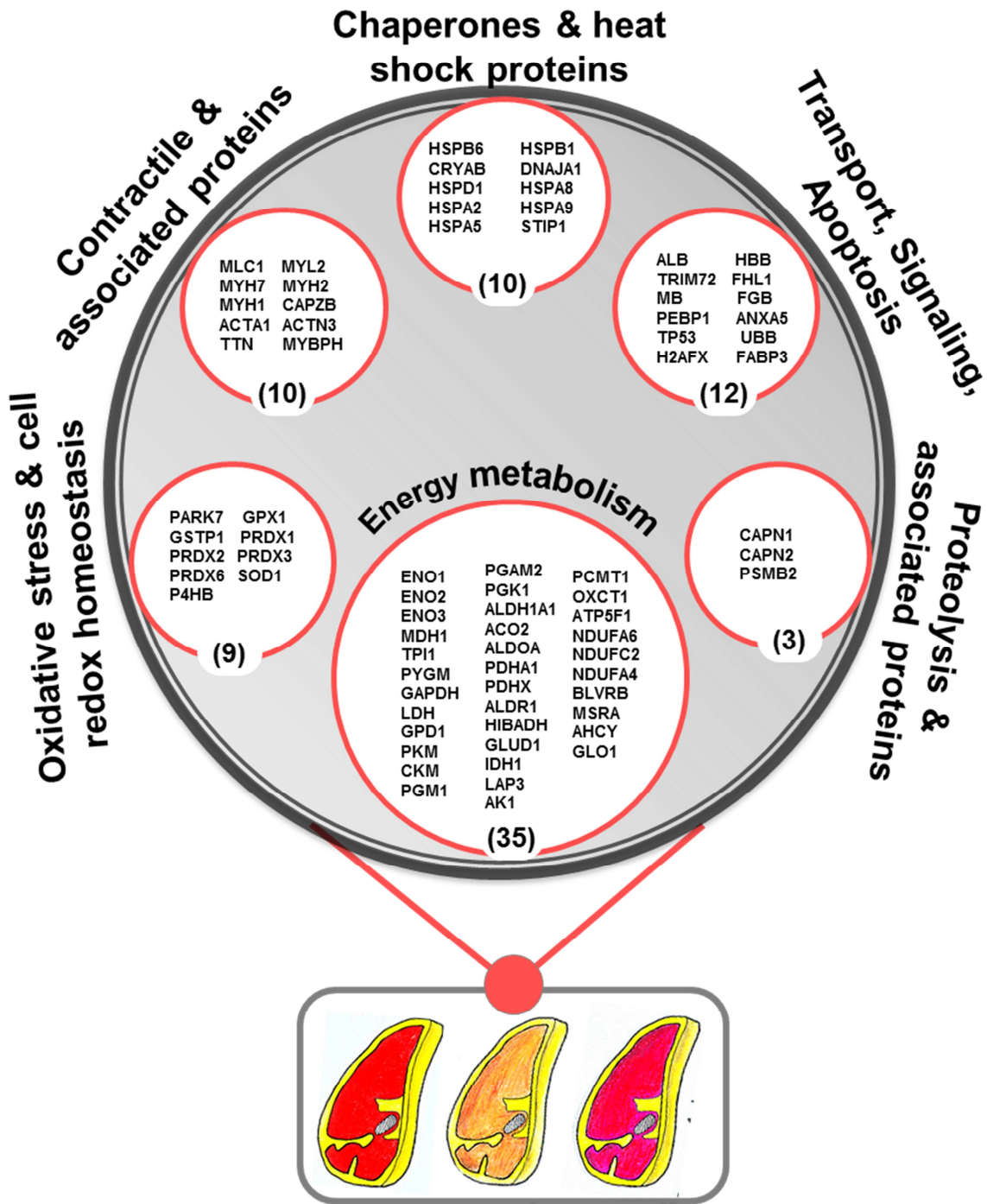
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Proteomic biomarkers of beef colour, *Trends in Foods Science & Technology*

Proteomic biomarkers of beef colour

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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 integrative 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), Phosphoglucosyltransferase 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 integrative work proposes a list of 27 putative biomarkers of beef colour for validation using adapted high-throughput methods.

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

45 1. Introduction

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

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

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

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

92 **2.1 Meat colour definition and measurement**

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

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

109

2.2 Colour versus colour stability

110

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

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

2.3. Mechanisms by which proteins may influence beef colour

130
131 As discussed above, the concentration of myoglobin (protein pigment) in a given muscle
132 determines the redness a^* -value and yellowness b^* -value of the meat surface, and also the L^* -
133 value. As animals mature, their muscles switch to being more aerobic, with higher levels of
134 myoglobin and also of oxidative enzymes, hence the muscle colour changes from the white to pale-
135 pink colour of veal to the bright/deep red colour of mature beef. The concentration of myoglobin
136 also varies between muscles. Hence the darker red colour of the muscles containing predominantly
137 slow-twitch, oxidative type I fibres which contain higher levels of myoglobin (e.g. *Psoas major*)
138 compared to the paler red colour of muscles containing predominantly fast-twitch glycolytic type
139 IIb fibres (e.g. *Longissimus lumborum*) which contain lower levels of myoglobin. Variations in
140 levels of myoglobin, oxidative enzymes and organelles (especially mitochondria) would therefore
141 be expected to be associated with variations in beef colour.

142 Conversely, the lightness of a meat surface, which is measured by the L^* , is mainly caused by
143 scattering of light from within the muscle structure as well as fluid on the surface (Purslow *et al.*
144 2020) and to a lesser extent by the concentration of the pigment myoglobin. Both light scattering
145 and surface fluid are generally greater in muscles with predominantly glycolytic fibre types, which
146 have a tendency to exhibit a pale, weepy quality defect called PSE in pork, and which also occurs in
147 beef muscle. These predominantly fast-twitch glycolytic muscles will not only have lower
148 concentrations of myoglobin and associated enzymes, but will also tend towards faster pH fall *post-*
149 *mortem* and lower ultimate pH, principally due to higher glycogen storage. The glycolytic rate
150 influences *post-mortem* changes to proteins such as myosin, actin, troponin, and some metabolic
151 proteins, particularly glycolytic enzymes in the sarcoplasm, and these *post-mortem* protein changes
152 can influence the ultimate meat quality. Conversely, oxidative slow twitch fibre types generally
153 have lower glycogen storage and tend towards having a high ultimate pH, which is associated with
154 lower L^* values, due to both reduced light scattering (Purslow *et al.* 2020) and higher oxygen
155 consumption in the surface. Thus, variations in levels of glycogen and in glycolytic and oxidative
156 enzymes would also be expected to be associated with variations in beef colour, but through a
157 different mechanism to myoglobin. Differences in contractile and metabolic properties, including
158 variations in pH, certainly contribute to the differences in both colour and colour stability of
159 different muscles, and different regions of muscles (Nair *et al.* 2018a). The principal causes of
160 changes in the achromatic aspects of meat colour are thought to be the lateral spacing of
161 myofilaments/myofibrils and the denaturation of sarcoplasmic proteins.

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

163 A computerized search using Pubmed.gov (NCBI), Google Scholar, Web of Science (Clarivate
164 Analytics) and Scopus databases was performed, attempting to identify all relevant published
165 proteomics studies dealing with meat colour. Databases were searched from August 2018 to January
166 2020 for studies published from 2000 to 2020. The keywords used were “proteomic”, “omic”,
167 “proteome”, “protein”, “biomarker” and “colour”, in combination with “meat” or “muscle”. There
168 were no language or data restrictions, but only proteomic studies using muscle samples (meat) were
169 considered. The literature search focused exclusively on full text articles published in peer-reviewed
170 journals to ensure the methodological quality of the studies.

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

176 Dry) meat as the mechanisms are different. Thirteen peer-reviewed research articles including those
177 using targeted proteomics (Kim *et al.* 2008; Joseph *et al.* 2012; Canto *et al.* 2015; Gagaoua *et al.*
178 2015; Wu *et al.* 2015; Nair *et al.* 2016; Wu *et al.* 2016; Gagaoua *et al.* 2017a; Gagaoua *et al.* 2017b;
179 Gagaoua *et al.* 2017c; Yu *et al.* 2017a; Gagaoua *et al.* 2018; Yang *et al.* 2018) fulfilled the criteria
180 and were used to create the database of beef colour biomarkers (**Table 1** and **Table S1**).

181 **4. Database description**

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

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

198 Different machines, different versions of machines and variation in the aperture size can
199 influence the colour measurements obtained (Warner 2014) although specifying a different
200 illuminant or observer angle within a machine, usually only has a negligible influence. Some of the
201 studies used a^* and b^* values to compute hue angle (h^*) to assess discoloration, and saturation
202 index (C^*) to evaluate red colour intensity (AMSA 2012). Other colour parameters reported in
203 some studies were; MRA, Metmyoglobin Reducing Activity, OCR, Oxygen Consumption Rate and
204 R630/580, surface colour stability. Ten studies that reported protein abundances, but not correlation
205 analyses, or comparisons of muscle proteome abundance with ageing time colour traits or
206 proteomics of dark cutting, were not included in the database (Yu *et al.* 2017b; Mahmood *et al.*
207 2018; Nair *et al.* 2018b; Zhai *et al.* 2018; Zhang *et al.* 2018; Hughes *et al.* 2019; Kim *et al.* 2019;

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

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

218 It should be noted that the selection criteria for proteins identified in the 13 papers differed
219 somewhat; eight papers selected proteins by 1.5-fold, or two-fold, or $P < 0.05$ differences in protein
220 abundance between treatments. The remaining five papers analysed the abundance of 21 – 29 pre-
221 selected protein biomarkers that had been identified in earlier studies. The highest number of
222 proteins were identified for the *Longissimus* muscle, due to its more frequent use, with 59 proteins
223 across the various colour traits, and 54 of them correlated with L^* , a^* or b^* (**Figure 1**). The ST
224 muscle followed with 27 proteins (25 correlated with L^* , a^* or b^*), the PM muscle with 19 proteins
225 (17 correlated with L^* , a^* or b^*), the RA muscle with 14 proteins (12 correlated with L^* , a^* or b^*)
226 and the SM muscle with 6 proteins, correlated with only MRA and R630/580 colour traits (**Table**
227 **1**). The Venn diagram illustrates the degree of overlap among the muscles by summarising the
228 similarities (common) and divergences (specific) within the proteins for the five muscles and for
229 each muscle (**Figure 1**).

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

240

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

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

251 The protein-protein interactions (interactomics) of biological function of the proteins from the
252 five muscles (**Figure 3A**) or LT muscle alone (**Figure 3B**) were analysed using the STRING
253 webservice database (<http://string-db.org/>). Default settings of confidence of 0.5 and 4 criteria for
254 linkage: Co-occurrence, experimental evidences, existing databases and text mining were used.
255 Considering the limitation of the GO annotation of genes in bovine, we converted their UniprotIDs
256 to orthologous human EntrezGene IDs using BioMart (<http://www.ensembl.org/biomart/>).
257 Functional annotation analysis of the 79 proteins was further checked using the PANTHER
258 classification system to identify ontology categories for the complete list of proteins and to identify
259 the main biological pathways. Among the 79 proteins we further searched for secreted proteins that
260 may be secreted in the extracellular environment and involved in interactions between cells at short
261 and long distances. Eight of such proteins (HSPB6, HSPA5, GAPDH, PARK7, PRDX6, P4HB,
262 ALB and FGB) were revealed by ProteINSIDE tool and were highlighted in the protein networks
263 (**Figure 3A,B**) by black and red ovals, for those secreted by classical pathways or non-classical
264 pathways, respectively.

265 6. Six Main biological pathways associated with beef colour

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

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

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

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

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

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

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

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

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

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

289 The distributions and molecular functions of the clustered groups of proteins of interest are
290 described for each muscle in **Table S4** and **Table S5**. The presence/absence of these proteins in the
291 13 studies is illustrated in **Figure S3**. Across the muscles, the most represented functions for the
292 five muscles taken together are: proteins involved in catalytic, metabolism and ATP metabolic
293 processes (44.3%, 35 proteins); binding, cofactor and transport proteins, signalling or apoptosis
294 (15.2%, 12 proteins); chaperones and heat shock proteins (12.7%, 10 proteins) and contractile and
295 associated proteins (12.7%, 10 proteins). Oxidative stress and cell redox homeostasis, and
296 proteolysis and associated proteins, represent 11.4% (9 proteins) and 3.8% (3 proteins),
297 respectively. Hence; irrespective of the muscle, the catalytic, metabolism and ATP metabolic
298 process pathway dominates (**Table S5**). Of the five muscles, *Longissimus* muscle was considered
299 representative (more interesting), and was chosen to illustrate the functional interaction network
300 (**Figure 5**). The lack of identified biological pathways in some muscles is probably due to low
301 numbers of identified proteins due to the lower representation of some muscles across the studies.

302

303

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

305 Of the 79 protein biomarkers, 27 were reported 3 to 8 times in independent studies, generally as
306 being correlated with beef colour traits. These similarities ranged from one protein common to 8
307 studies to 13 proteins identified in at least 3 studies (**Table 1** and **Figure S3**). At the top of this list,
308 β -enolase (ENO3) was correlated with colour traits in 8 studies (Joseph *et al.* 2012; Gagaoua *et al.*
309 2015; Nair *et al.* 2016; Wu *et al.* 2016; Gagaoua *et al.* 2017a; Gagaoua *et al.* 2017b; Gagaoua *et al.*
310 2017c; Yu *et al.* 2017b); Peroxiredoxin 6 (PRDX6) (also found as a secreted protein) in 7 studies
311 (Gagaoua *et al.* 2015; Wu *et al.* 2015; Wu *et al.* 2016; Gagaoua *et al.* 2017a; Gagaoua *et al.* 2017b;
312 Gagaoua *et al.* 2017c; Yang *et al.* 2018); HSP27 (HSPB1, also identified as a secreted protein) in 6
313 studies (Kim *et al.* 2008; Joseph *et al.* 2012; Wu *et al.* 2016; Gagaoua *et al.* 2017a; Gagaoua *et al.*
314 2017b; Gagaoua *et al.* 2017c); and 5 studies found that Phosphoglucosmutase 1 (PGM1), Superoxide
315 Dismutase (SOD1) and Calpain-1 catalytic subunit or μ -calpain (CAPN1) were related to colour
316 (**Table 1** and **Figure S3**). Eight other proteins: HSP40 (DNAJA1), HSP70-8 (HSPA8), HSP70-
317 Grp75 (HSPA9), Malate dehydrogenase 1 (MDH1), Triosephosphate isomerase 1 (TPI1), Lactate
318 dehydrogenase (LDH), Myosin-7 (MYH7) and Myosin-2 (MYH2) were reported 4 times and 13
319 were reported 3 times: HSP20 (HSPB6), α B-crystallin (CRYAB), HSP72 (HSPA2), Pyruvate
320 kinase M 2 (PKM), Creatine kinase M type (CKM), Fructose-bisphosphate aldolase A (ALDOA),
321 DJ-1 (PARK7), Peroxiredoxin 2 (PRDX2), Myosin light chain 1 (MLC1), Myosin-1 (MYH1), α -
322 Actin, (ACTA1), Myosin binding protein-H (MYBPH) and Phosphatidylethanolamine-binding
323 protein 1 (PEBP1).

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

329 7.1. Protein biomarkers belonging to the catalytic, metabolism & ATP metabolic pathway

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

337 correlations occurs when the biochemical mechanism underlying the correlation has a major
338 contribution to the studied phenomenon but other factors are also involved. The multifactorial and
339 interdependent character of the influence of *post-mortem* metabolism and oxidative properties on
340 meat colour is well known and may explain the inversion of correlations observed. For example,
341 lactate, produced under the anaerobic conditions of glycogen breakdown in the *post-mortem*
342 muscle, may influence the redox status of the muscle, which may impact meat colour (Joseph et al.
343 2012). Regeneration of ATP depends on glycogen breakdown and is correlated to the extent of pH
344 decline (Robergs *et al.* 2004), which can also influence colour, both from a myoglobin status and
345 from a structural perspective. Metabolism of ATP is the key determinant of the rate and extent of
346 pH fall *post-mortem* which, through its effect of decreasing myofilament lattice spacing and
347 myofibril diameter, has a contribution to increasing values of L^* through the achromatic processes
348 of light scatter (Hughes *et al.* 2019). Some proteins of this group reported in **Table 1**, highlight that
349 an increase in protein abundance is associated with a decrease in L^* . This observation suggests that
350 the increased amounts of some glycolytic enzymes are largely not affecting L^* values through this
351 achromatic mechanism.

352 **7.1.1. ENO3, a robust biomarker irrespective of muscle and colour parameter**

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

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

371 ENO3 participates in multi-enzyme complexes present on the sarcomere, possibly in association
372 with other proteins (**Figure 3A,B**) involved in colour, such as heat shock proteins (Wulff et al.
373 2012) and contractile proteins (Hughes *et al.* 2014; Hughes *et al.* 2019). In keeping with this, Nair
374 *et al.* (2018a) comparing the inside colour-labile and the outside colour-stable regions of the
375 *Semimembranosus* (SM) muscle, found differences in the sarcoplasmic proteome, including ENO3.
376 They suggest that differences in the contractile and metabolic properties including variations in pH,
377 contribute to the differences in colour stability of the two regions (Nair et al. 2018a).

378 **7.1.2. PGM1, TP11, LDH and PKM are glycolytic enzymes and are biomarkers for beef meat** 379 **colour, irrespective of the contractile and metabolic properties and colour-stability of muscles**

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

387 The relationships between PGM1 and several colour traits from different muscles and animal
388 types are consistent with the knowledge that glycolysis during early *post-mortem* period affects
389 many meat quality properties including tenderness and colour (Anderson *et al.* 2014). First, hypoxic
390 conditions occurring in the *post-mortem* muscle, increase the abundance and the activity of PGM1,
391 which at least partly regulates the *post-mortem* balance between G-1-P and G-6-P. The latter may
392 be associated to meat colour, through the effect on pH decline. For example, metabolite profiles,
393 including G-6-P in samples collected during the early *post-mortem* period (Yu *et al.* 2019) differed
394 amongst muscles which were subsequently found to differ in colour stability during display
395 (Abraham *et al.* 2017). Second, PGM1 undergoes posttranslational modifications through
396 phosphorylation, acetylation or methylation (Anderson et al. 2014) and the direction of associations
397 of PGM1 with various colour parameters could be influenced by its isoforms (Anderson *et al.*
398 2014). Earlier studies suggested that according to muscle, stress level of the animal or the use of
399 *post-mortem* electrical stimulation, different PGM1 isoforms correlate with different meat quality
400 indicators (Laville *et al.* 2009; Bjarnadottir *et al.* 2010; Anderson *et al.* 2014). At relatively high
401 levels of G-1-P, greater activity of PGM1 increases the rate of its conversion into G-6-P, which,
402 depending on the energy requirements of the cell is either used in the glycolytic pathway, or for
403 regeneration of NADH; the latter being an important determinant of meat colour stability (Mancini
404 & Hunt 2005; Mitacek *et al.* 2019). Hence, depending on the G-1-P and G-6-P balance, the levels of

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

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

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

426 Pyruvate kinase M 2 (PKM) has four isoforms, two of which, PKM1 and PKM2, predominate in
427 skeletal muscle. In three of the studies (Canto *et al.* 2015; Wu *et al.* 2015; Wu *et al.* 2016) PKM2
428 was negatively correlated with colour parameters in the ST and PM muscles, similar to the TPI1
429 and PGM1 enzymes, and, depending on the muscle, in different directions for *Longissimus* muscle
430 for a^* , MRA and R630/580 parameters (**Table 1** and **Figure 5**). PKM catalyzes the last step of
431 glycolysis, the dephosphorylation of phosphoenolpyruvate to pyruvate and a greater PKM
432 abundance is likely to reflect a potentially greater production of pyruvate (**Figure 4**). Pyruvate is
433 transported into the mitochondria and favours the regeneration of NADH (Ramanathan & Mancini
434 2010). Muscle type and many other factors influence the glycolytic capacity of muscles which is
435 consistent with the differences obtained between studies (**Table S1**).

436 **7.1.3. ALDOA, a negative biomarker in colour-stable beef muscles**

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

457 **7.1.4. CKM, a positive biomarker in colour-stable beef muscles**

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

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

472 **7.1.5. MDH1, an oxidative enzyme biomarker of colour-stable beef muscles**

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

482 **7.2. Protein biomarkers belonging to chaperones & heat shock proteins (HSPs)**

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

494 HSPs have been proposed as regulators of apoptosis onset during the conversion of muscle to
495 meat (Sayd *et al.* 2006), consequently affecting proteolysis and meat qualities (Lomiwes *et al.*
496 2014a; Lomiwes *et al.* 2014b; Picard & Gagaoua 2020). Three small HSPs (HSPB1, HSPB6 and
497 CRYAB), one co-chaperone (DNAJA1) and 3 large 70kDa HSPs (HSPA8, HSPA9 and HSPA2)
498 were identified as major putative markers of beef colour (**Table 1** and **Figure 5**). Other studies on
499 beef colour proteomics also identified these same HSPs to be associated with colour in beef (Yu *et al.*
500 *et al.* 2017b; Mahmood *et al.* 2018; Zhang *et al.* 2018; Kim *et al.* 2019; Wu *et al.* 2019; Wu *et al.*
501 2020).

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

510 **7.2.1. HSPB1 is the top biomarker of beef colour among the small HSPs**

511 HSP27 (HSPB1) is the only small HSP correlated to colour traits (L^* , a^* , b^* , C^* and R630/580)
512 in two muscles (*Longissimus* and RA) across 6 studies (Kim *et al.* 2008; Joseph *et al.* 2012; Wu *et al.*
513 *et al.* 2016; Gagaoua *et al.* 2017a; Gagaoua *et al.* 2017b; Gagaoua *et al.* 2017c). The correlations with
514 all colour traits were positive in the RA muscle and in different directions in the *Longissimus*
515 muscle depending on the colour trait and animal type/breed (**Table 1** and **Figure 5**). Two other
516 studies also reported this protein to be related to beef colour (Mahmood *et al.* 2018; Kim *et al.*
517 2019).

518 The positive and negative relationships observed in the *Longissimus* may be related to
519 differences in the pre-slaughter stress level of the animal and/or the type of HSP27 isoform
520 involved (Ouali *et al.* 2013; Picard & Gagaoua 2020). In skeletal muscle, HSPB1 as well as HSPB6
521 and CRYAB protect against ischemia, hypertensive stress, and metabolic dysfunction (Dreiza *et al.*
522 2010). They have the capacity to bind to myofibrils (Lomiwes *et al.* 2014b), thereby protecting
523 skeletal muscle through structural protein complexes. For example, increased CRYAB levels were
524 associated with delayed myofibril degradation in beef with ultimate pH < 5.7. Their role in
525 protection against stress-induced denaturation of muscle proteins may partly explain their
526 relationship with meat colour (Gagaoua *et al.* 2015; Gagaoua *et al.* 2017c). Particularly, the
527 prevention of denaturation of sarcoplasmic proteins and myosin, would affect reflectance, light
528 scattering and myoglobin, thereby influencing all colour coordinates (Hughes *et al.* 2014; Hughes *et al.*
529 *et al.* 2019; Purslow *et al.* 2020). For example, through their action, the reduction of light scattering
530 would result in a lower L^* as developed in the following sub-section. Other effects may be related
531 to their ability to influence the redox status of the cell. For example, an increase in HSPs content
532 has been associated with lower oxidative stress levels of the cells, as indicated by lower levels of
533 thiobarbituric acid reactive species (Jammes *et al.* 2009).

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

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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.

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7.3. Protein biomarkers belonging to contractile & associated proteins

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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 myofibrillar 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.

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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 & Oliván 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

601 positive for the RA muscle (**Figure 5**). The PLA2 (phospholipase A2) of PRDX6 is believed to be
602 activated during cellular stress and triggers ROS production; the enhanced oxidative activity
603 increases contractile function (Gong *et al.* 2006). The positive relationship between Peroxiredoxins
604 and redness, shown by the positive correlations between PRDX6, PRDX1, PRDX2 and PRDX3 and
605 a^* -values in the *Longissimus* muscle, may be related to their different functions. First, they may act
606 as antioxidants, protecting OxyMb from attacks by peroxides (Wang *et al.* 2003). Second,
607 peroxiredoxins may inhibit lipid oxidation, and consequently myoglobin oxidation (Faustman *et al.*
608 2010; Joseph *et al.* 2012). Finally, PRDX6 may counteract processes underlying meat discoloration
609 by competing with myoglobin for oxygen or products of oxidative stress reactions. PRDX6 is
610 further implicated in a variety of cellular processes, including metabolism, apoptosis and ageing
611 (Fisher 2017) and was proposed as a potential biomarker of beef tenderness (Jia *et al.* 2009;
612 Guillemin *et al.* 2011; Picard & Gagaoua 2020).

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

619 **7.4.2. SOD1, a biomarker regardless of the colour-stability of muscles**

620 Superoxide dismutase (SOD1), was correlated in 5 studies with meat colour traits (Gagaoua *et*
621 *al.* 2015; Wu *et al.* 2016; Gagaoua *et al.* 2017b; Gagaoua *et al.* 2017c; Gagaoua *et al.* 2018) and in
622 2 muscles (**Table 1** and **Figure 1**). Correlations were positive for the PM muscle and in the
623 opposite direction for a^* and b^* colour coordinates of the *Longissimus* muscle (**Figure 5**). SOD1 is
624 a member of a ubiquitous family of metalloenzymes that eliminate excess ROS, thus preventing
625 damage caused by free radicals in cells (Vacek *et al.* 2010). It allows fast dismutation of O_2^- to O_2
626 and H_2O_2 . SOD1 has been identified as a biomarker of meat toughness (Grabez *et al.* 2015;
627 Malheiros *et al.* 2019; Picard & Gagaoua 2020).

628 **7.4.3. DJ-1 (PARK7), a negative biomarker of colour-stable beef muscles**

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

634 DJ-1 has a key role in scavenging mitochondrial H_2O_2 and limiting mitochondrial fragmentation.
635 During oxidative stress, DJ-1 is re-localized from the cytosol to the mitochondria (Junn *et al.* 2009).
636 As indicated above, due to their role in oxygen consumption and their reducing capacity,
637 mitochondria play a major role in meat colour (Ramanathan & Mancini 2018). It is therefore likely
638 that DJ-1 plays a role in meat colour via mitochondria protection, probably by interacting with other
639 pathways, particularly heat shock proteins (Wu *et al.* 2020) and energy metabolism (Picard &
640 Gagaoua 2020). DJ-1 has also been shown to be related to beef tenderness (Jia *et al.* 2007; Jia *et al.*
641 2009; Mahmood *et al.* 2018; Malheiros *et al.* 2019; Picard & Gagaoua 2020).

642 **7.5. CAPN1 and PEBP1 as protein biomarkers of beef colour**

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

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

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

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

668 **8. Conclusion and future perspectives**

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

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

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

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

701 **Declaration of competing interest**

702 The authors declared that there is no conflict of interest.

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934

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

Table 1. Continued.

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

Table 1. Continued.

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

Table 1. Continued.

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

Table 1. Continued.

		Redness (a^*)	L	-	Gagaoua et al. 2015
		Yellowness (b^*)	L	-	Gagaoua et al. 2017b
		Redness (a^*)	L	+	Gagaoua et al. 2018a
Superoxide Dismutase (<i>SOD1</i>)	P00441	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 (<i>P4HB</i>)	P07237	Redness (a^*)	PM	-	Wu et al. 2016
Contractile & associated proteins					
		Redness (a^*)			Canto et al. 2015
Myosin light chain 1 (<i>MLC1</i>)	Q15049	Yellowness (b^*)	L	+	Gagaoua et al. 2017b
		Yellowness (b^*)			Gagaoua et al. 2017c
		Chroma (C^*)			Gagaoua et al. 2017c
Myosin regulatory light chain 2, (<i>MYL2</i>)	P10916	Redness (a^*)	L	+	Canto et al. 2015
		R630/580			
		Redness (a^*)	ST	-	Yu et al. 2017a
Myosin-7 (<i>MYH7</i>)	P12883	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
		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
Myosin-2 (<i>MYH2</i>)	Q9UKX2	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
		Lightness (L^*)	RA	+	Gagaoua et al. 2017a
Myosin-1 (<i>MYH1</i>)	P12882	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 (<i>CAPZB</i>)	P47756	Redness (a^*)	L	-	Gagaoua et al. 2017b
		Chroma (C^*)			Gagaoua et al. 2017b
		Lightness (L^*)	L	-	Gagaoua et al. 2015
Actin, alpha skeletal muscle (<i>ACTA1</i>)	P68133	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 (<i>ACTN3</i>)	Q0III9	Yellowness (b^*)	L	-	Gagaoua et al. 2018a
		Lightness (L^*)			
Titin (<i>TTN</i>)	Q8WZ42	Redness (a^*)	L	+	Gagaoua et al. 2018a
		Yellowness (b^*)		+	
		Chroma (C^*)		+	
		Lightness (L^*)	L	+	Gagaoua et al. 2015
Myosin binding protein-H (<i>MYBPH</i>)	Q13203	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 (<i>CAPN1</i>)	P07384	Lightness (<i>L</i> *)	L	-	Gagaoua et al. 2015
		Yellowness (<i>b</i> *)	L	+	Gagaoua et al. 2015
		Chroma (<i>C</i> *)	L	+	Gagaoua et al. 2015
		Redness (<i>a</i> *)	RA	+	Gagaoua et al. 2017a
		Chroma (<i>C</i> *)	RA	+	Gagaoua et al. 2017a
		Yellowness (<i>b</i> *)	L	+	Gagaoua et al. 2017b
		Yellowness (<i>b</i> *)	L	-	Gagaoua et al. 2018a
		Hue angle (<i>h</i> *)	L	-	Gagaoua et al. 2018a
		Lightness (<i>L</i> *)	L	+	Gagaoua et al. 2017c
		Chroma (<i>C</i> *)	L	+	Gagaoua et al. 2017c
Calpain-2 catalytic subunit (<i>CAPN2</i>)	P17655	Lightness (<i>L</i> *)	L	+	Gagaoua et al. 2017c
		Chroma (<i>C</i> *)			
Proteasome subunit beta type-2 (<i>PSMB2</i>)	P49721	Redness (<i>a</i> *)	ST	-	Yu et al. 2017a
Binding, cofactor & transport proteins, signaling or apoptosis					
Serum albumin (<i>ALB</i>)	P02768	Redness (<i>a</i> *)	ST		Wu et al. 2015
		MRA	ST		Wu et al. 2015
		Redness (<i>a</i> *)	PM	-	Wu et al. 2016
		MRA	PM		Wu et al. 2016
Hemoglobin subunit beta (<i>HBB</i>)	P68871	Redness (<i>a</i> *)	ST	+	Yu et al. 2017a
Tripartite motif-containing protein 72 (<i>TRIM72</i>)	Q6ZMU5	Lightness (<i>L</i> *)	L	-	Gagaoua et al. 2018a
Four and a half LIM domains 1 (<i>FHL1</i>)	Q13642	Lightness (<i>L</i> *)		-	
		Redness (<i>a</i> *)	L	+	Gagaoua et al. 2018a
		Chroma (<i>C</i> *)		+	
Myoglobin (<i>MB</i>)	P02144	Redness (<i>a</i> *)	L	-	
		MRA	L	-	Wu et al. 2016
		Redness (<i>a</i> *)	PM	+	
		MRA	PM	+	
Fibrinogen beta chain (<i>FGB</i>)	P02675	Redness (<i>a</i> *)	ST	+	Yu et al. 2017a
Phosphatidylethanolamine-binding protein 1 (<i>PEBP1</i>)	P30086	Redness (<i>a</i> *)	L	-	Canto et al. 2015
		R630/580	L	-	Canto et al. 2015
		Redness (<i>a</i> *)	L	+	Wu et al. 2016
		MRA	SM	-	Nair et al. 2016
Annexin A5 (<i>ANXA5</i>)	P08758	MRA	ST		Wu et al. 2015
		Redness (<i>a</i> *)	PM	-	Wu et al. 2016
Cellular tumor antigen p53 (<i>TP53</i>)	P04637	Lightness (<i>L</i> *)			Gagaoua et al. 2017b
		Hue angle (<i>h</i> *)	L	+	Gagaoua et al. 2017b
		Yellowness (<i>b</i> *)			Gagaoua et al. 2017c
Polyubiquitin-B (<i>UBB</i>)	P0CG47	Redness (<i>a</i> *)	L	+	Wu et al. 2016
		MRA			
Histone H2AX (<i>H2AFX</i>)	P16104	Yellowness (<i>b</i> *)	L	+	Gagaoua et al. 2017c
		Hue angle (<i>h</i> *)			
Fatty acid-binding protein (<i>FABP3</i>)	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 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, BLVRR, 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.

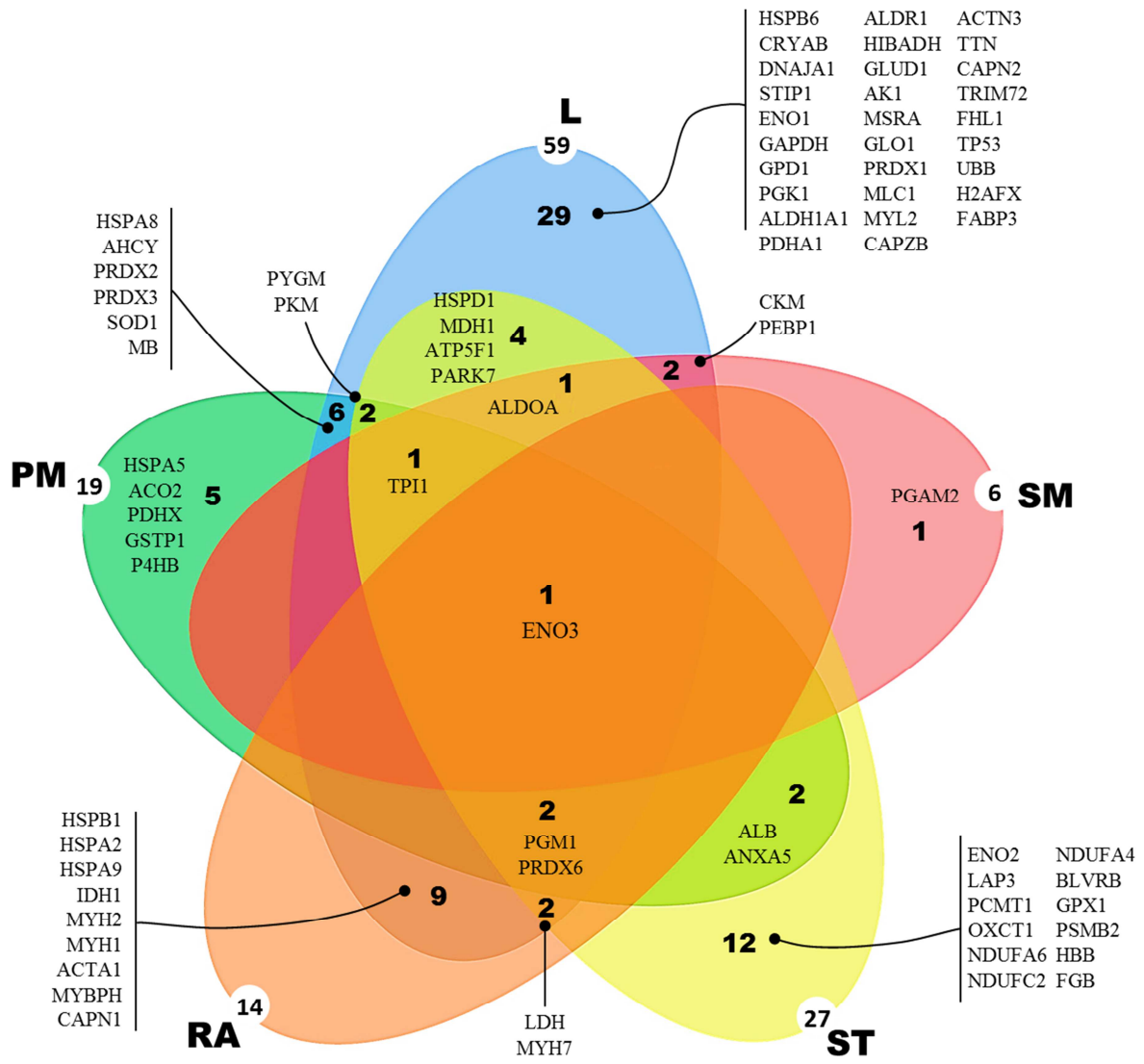
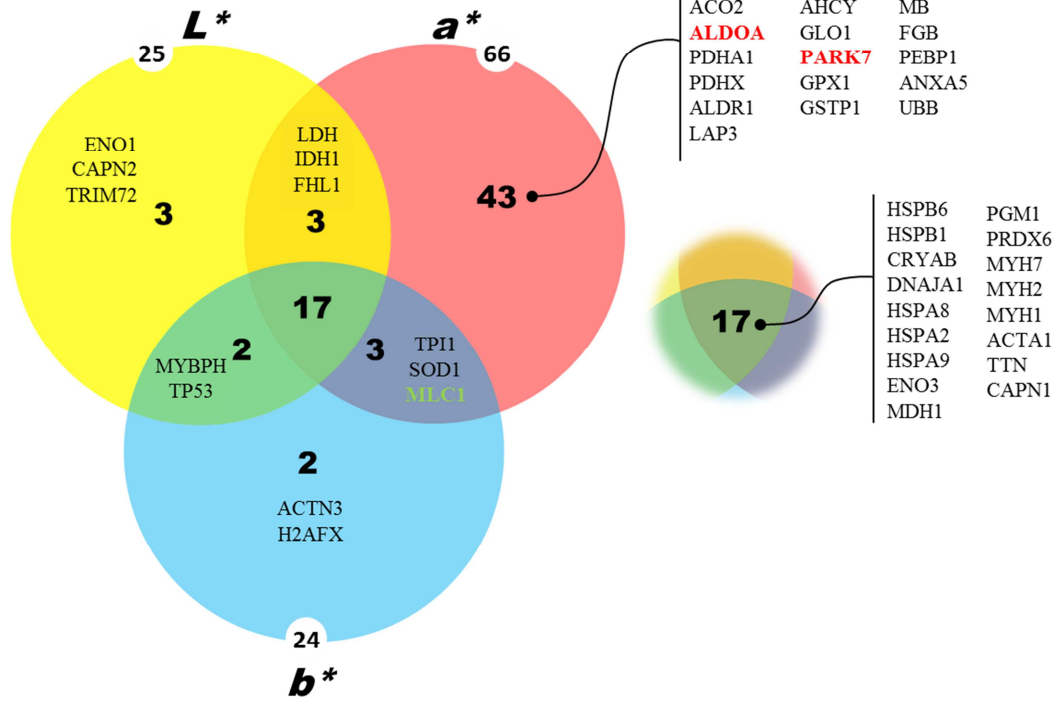


Figure 1.

A



B

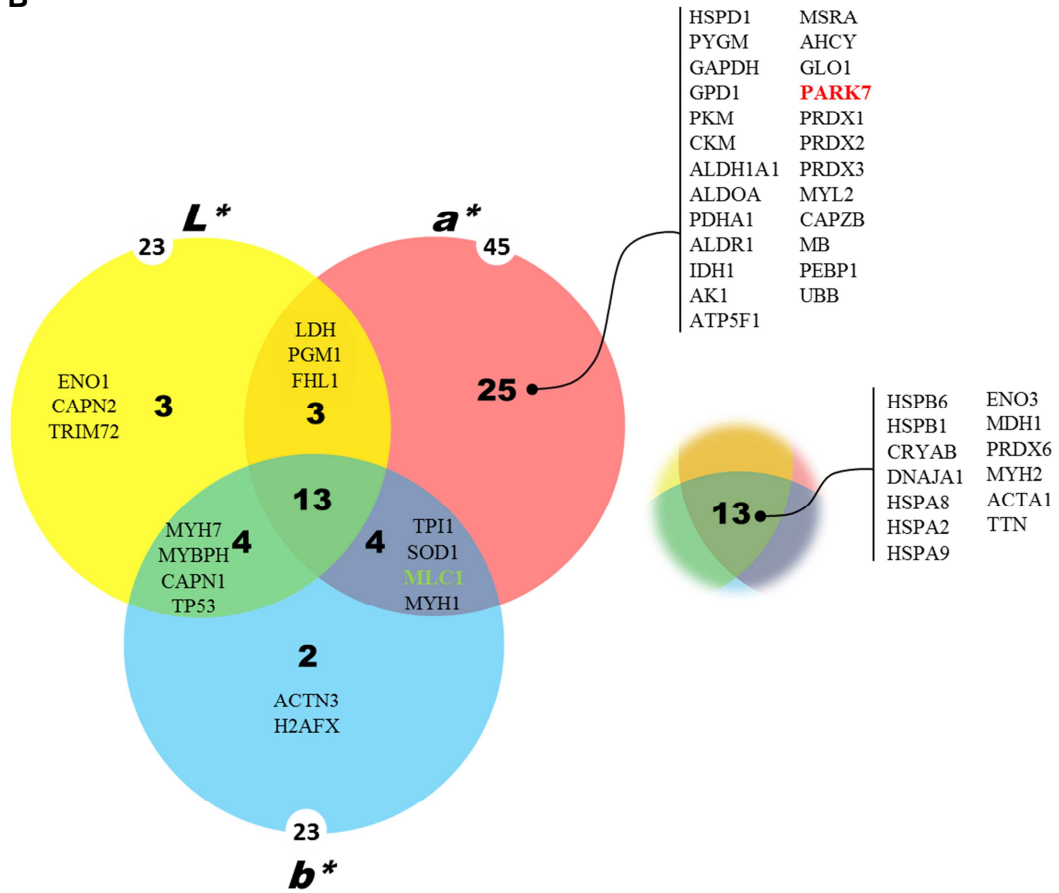


Figure 2.

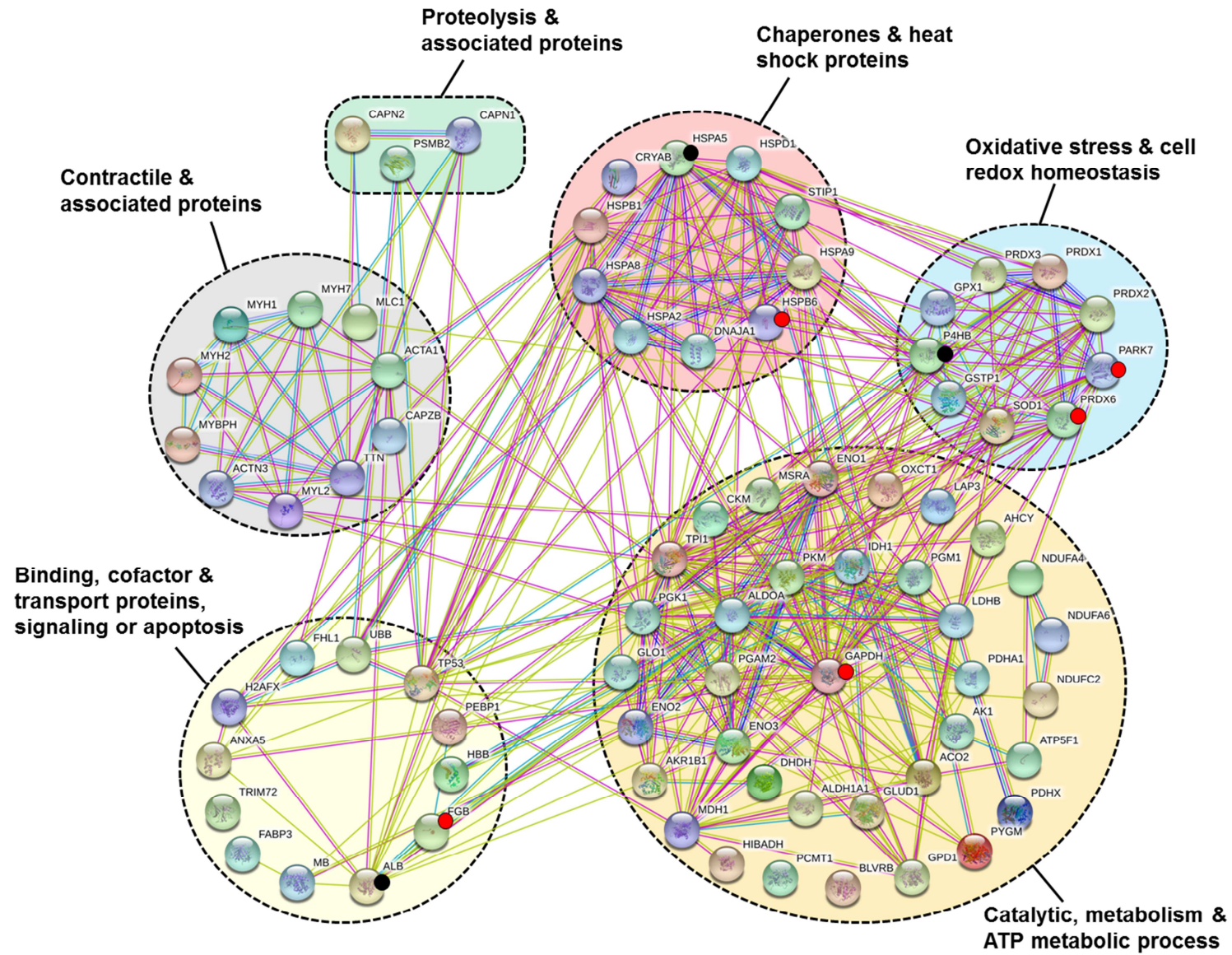


Figure 3A.

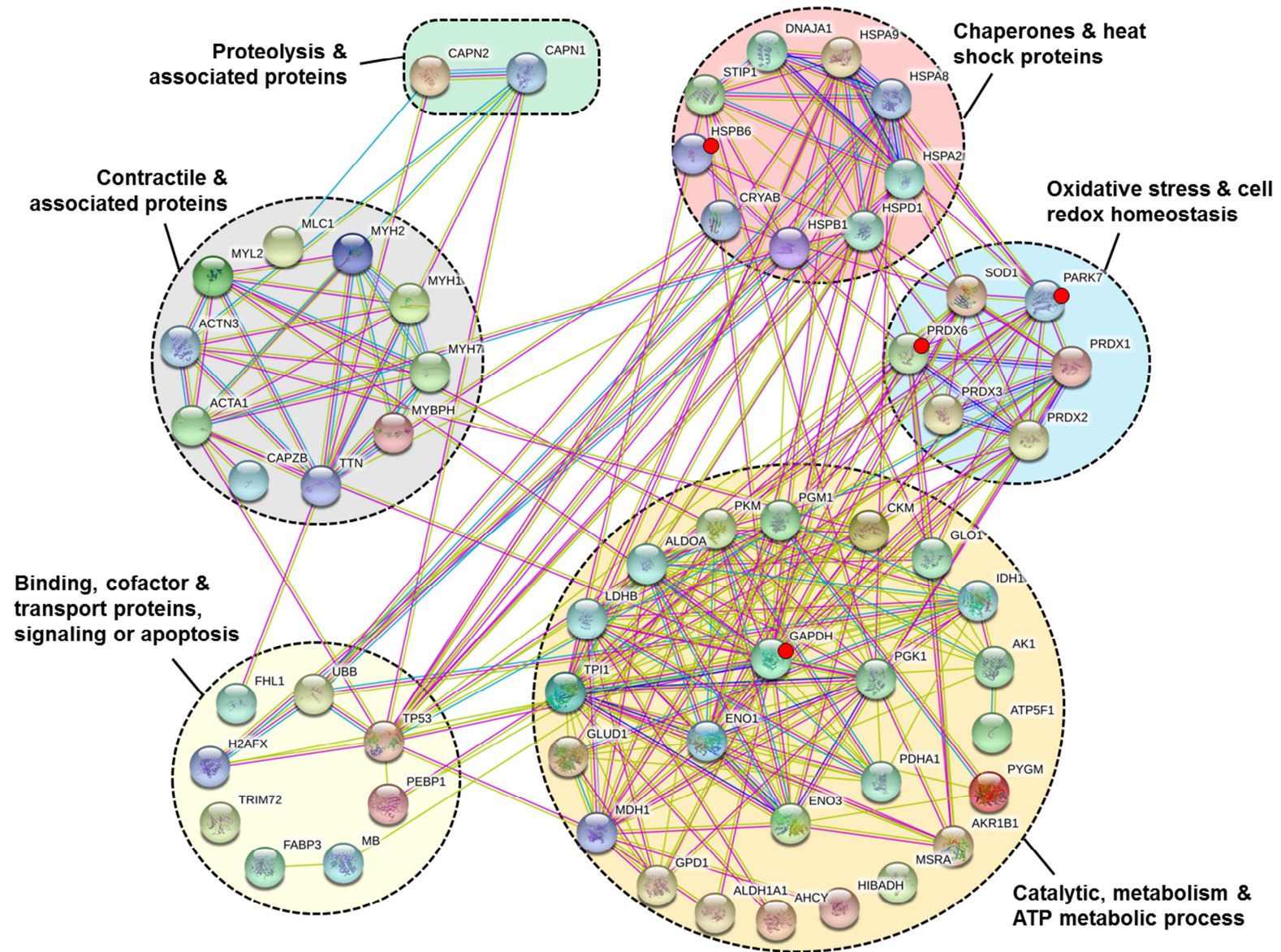


Figure 3B.

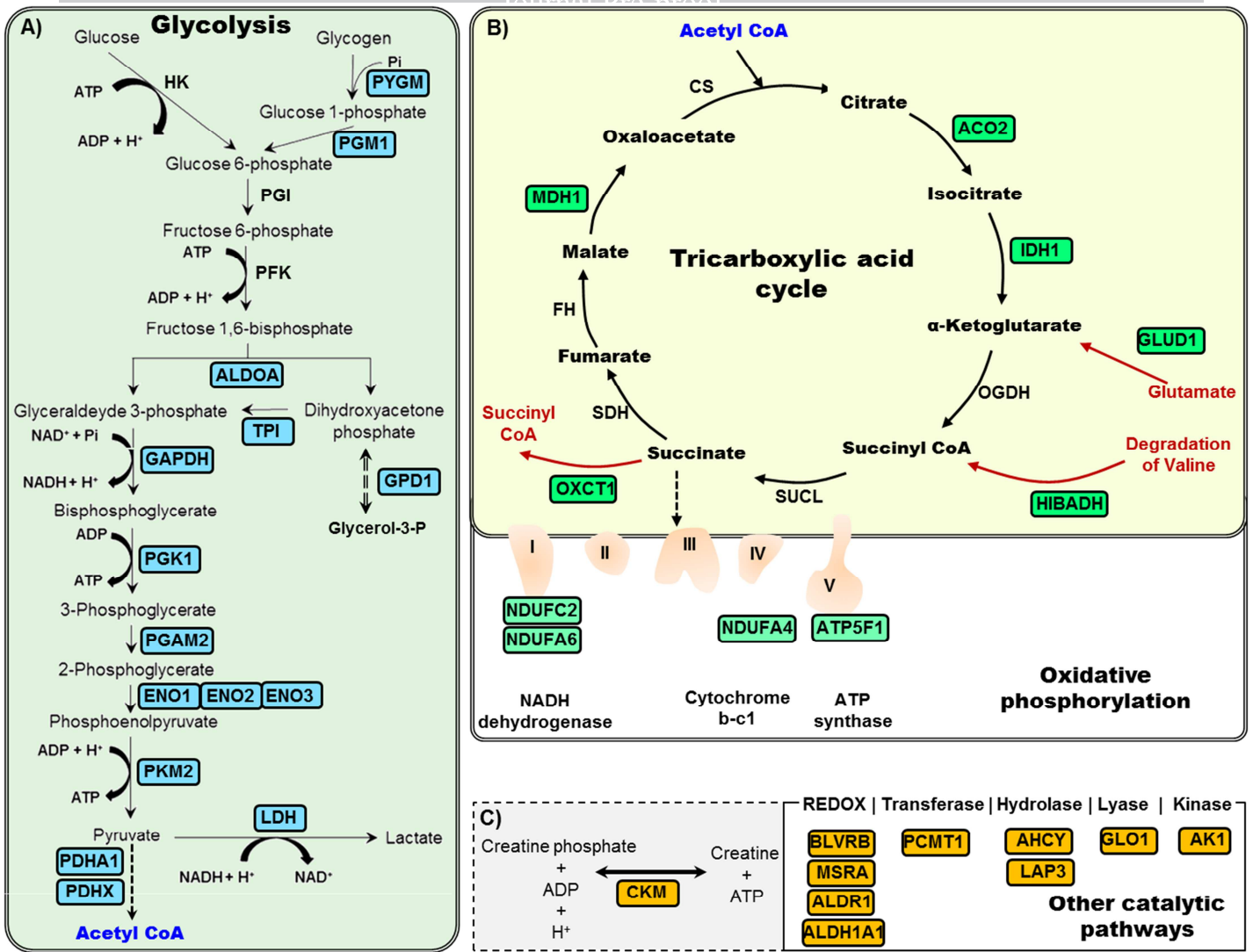


Figure 4.

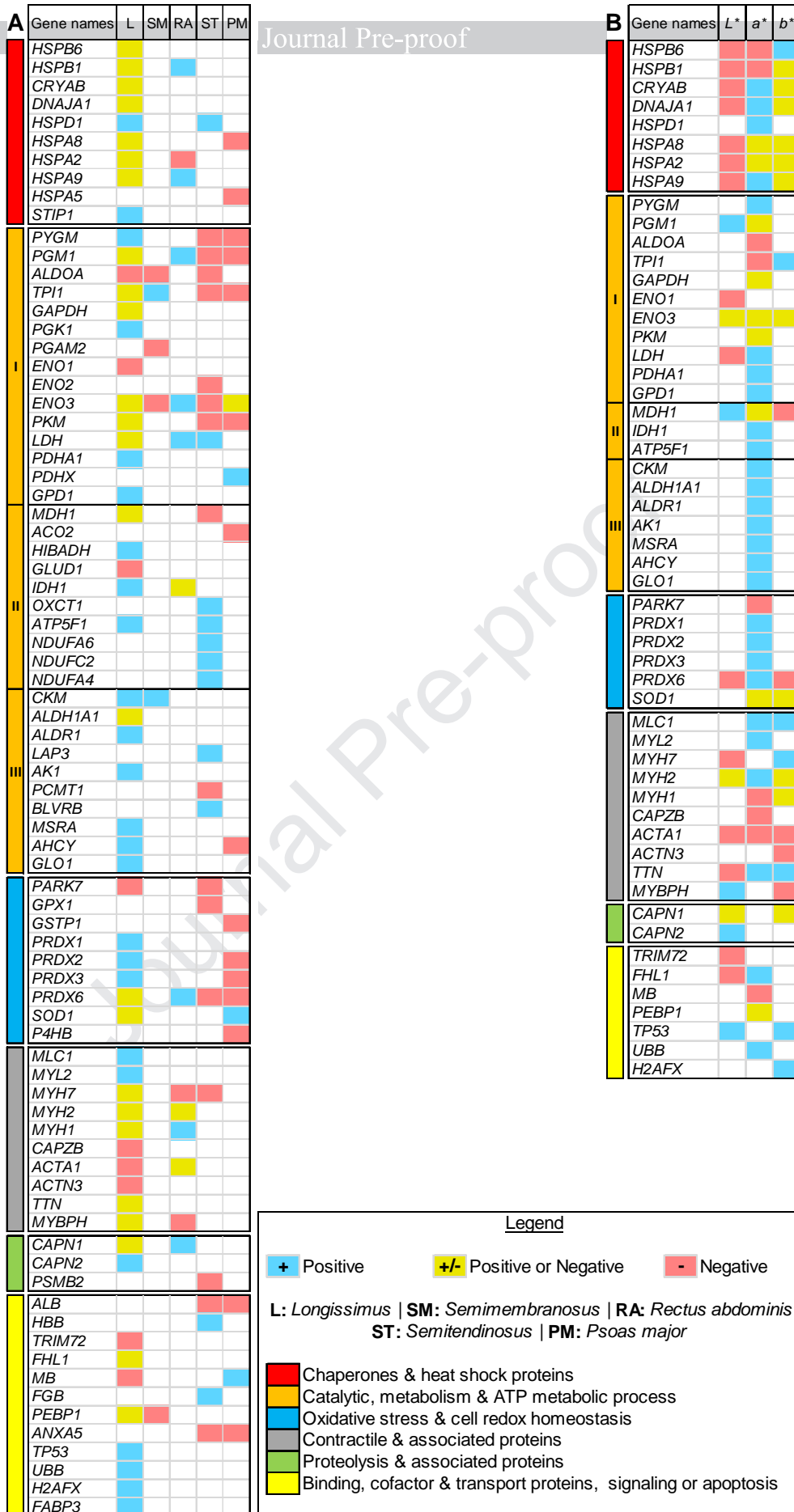


Figure 5.

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 -enolase (ENO3) is a generic biomarker irrespective of muscle type and colour trait

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