PROTEOMIC BIOMARKERS OF MEAT COLOUR OF BLONDE D'AQUITAINE YOUNG BULLS: TOWARDS A BETTER COMPREHENSION OF THE BIOLOGICAL MECHANISMS

Mohammed Gagaoua[1,3], E. M. Claudia Terlouw[1,2], Didier Micol[1,2], Abdelghani Boudjellal[3], Jean-François Hocquette[1,2], Brigitte Picard [1,2]

[1] INRA, UMR 1213 Herbivores, F-63122 Saint-Genès- Champanelle, France
[2] Clermont Université, VetAgro Sup, UMR 1213 Herbivores, B.P. 10448, F-63000 Clermont-Ferrand, France
[3] Equipe Maquav, INATAA, Université des Frères Mentouri Constantine 1, Route de Ain El-Bey, 25000 Constantine, Algeria

Abstract – Relationships with CIE-*L*a*b colour traits of protein biomarkers known to be related to tenderness were studied in *Longissimus thoracis* muscles from 21 French Blonde Aquitaine young bulls. *L*, a** and *b** coordinates were correlated with 9, 5 and 8 of the 21 biomarkers, respectively. Regression models explained between 47 and 65% of the variability between individuals in *L*a*b** values. Results suggest that development of colour and tenderness may share common biological pathways. They further show that inducible Hsp70s and µ-calpaïn influence the three colour parameters interactively.**

Key Words – beef meat, biomarkers, colour, biological mechanisms.

I. INTRODUCTION

Meat industry needs to predict and control meat quality to guarantee good quality to consumers. Over the last decade, a number of biomarkers were found to be related to the underlying mechanisms which determine tenderness and which may help increase the economic value of meat [1]. These biomarkers are representative of several biological functions and were recently used to propose prediction equations of meat sensory qualities [2, 3]. Among meat quality characteristics, colour appears important in dictating meat purchase decisions [4]. The present work provides new insights in mechanisms involved in the determinism of the colour of meat of young bulls of the Blonde d'Aquitaine (BA) breed, using the above mentioned biomarkers. The BA breed produces meat of good quality but with a light colour which may hamper purchase by consumers.

II. MATERIALS AND METHODS

This study used 21 young bulls of the BA breed finished over two consecutive years (two replicated groups). At 12 months of age, they were subjected to a 105 day finishing period until slaughter. Diets consisted of concentrate (75%) and straw (25%). Animals were slaughtered at about 500 days of age at a live weight around 635 kg. They were slaughtered at the experimental abattoir of the INRA Research centre in compliance with the current ethical guidelines for animal welfare. *Longissimus thoracis* (LT) samples were excised from the 6th rib, 30 min after slaughtering for Dot-Blot analysis. Instrumental meat colour measurements (24h *post-mortem*) for lightness (*L**), redness (*a**) and yellowness (*b**) were recorded directly on the muscle tissue using a Minolta Chroma meter (model CR-300), equipped with a 0° viewing angle. Freshly cut 2.5 cm thick slices of muscle overwrapped on a polystyrene tray were used. Before measurement, the samples were refrigerated (1°C) for at least 1h. The Chroma meter was regularly calibrated using its standard white calibration tile (Y = 93.58, x = 0.3150, y = 0.3217). Three replicate measurements were taken and an average value was used for analysis. For Dot-Blot, samples were immediately frozen in liquid nitrogen following sampling and stored at -80°C until protein extraction. Total protein extractions were performed according to the protocol of Bouley *et al.* [5]. The abundance of 18 proteins [3] was quantified by Dot-Blot according to Guillemin *et al.* [6] using specific antibodies and expressed as arbitrary units. Three other biomarkers corresponded to myosin heavy chains were determined according to Picard *et al.* [7] using an adequate SDS-PAGE and expressed in percentage (%). For subsequent statistical analyses except descriptive statistics, all data were standardized for replicate using the Proc Standard of SAS 9.2 to obtain Z-scores. Multiple regression analyses were carried out using colour parameters as dependent variables and the 21 protein biomarkers as independent variables following the procedure recently described by [3]. Pearson correlation coefficients were generated from the Proc Corr of SAS. PCA analyses for each colour parameter were carried out using all significant correlated variables.

III. RESULTS AND DISCUSSION

Mean and standard deviations of colour parameters, and protein biomarkers abundances are displayed in Table 1. Although no information was available in the literature for Blonde d'Aquitaine meat colour, mean values of L^* a^* b^* were within the range of values reported for LT muscles from young bulls of breeds producing relatively light meat [8]. The three colour parameters were not correlated (r < 0.29). L^* a^* b^* values were correlated with various biomarker abundances ($P < 0.05$) and these were introduced in the PCAs (Fig. 1a – c). Hsp70-1A/B and μ-calpain were correlated with the three colour traits: negatively with L^* and positively with a^* and b^*. Seven common correlations were found for L^* and b^*: they were correlated with αB-crystallin, Hsp70-1A/B, Hsp70-8, MyHC-I, μ-calpain, Enolase 3 and MyBP-H (Fig. 1a and c), although directions differed. Hsp40 was positively correlated with both a^* and b^*. Actin (negatively) and MyHC-IIa (positively) were correlated with L^* only. Prdx6 and MDH1 were positively correlated with a^* only. Recent proteomic studies on the colour of pork, beef and fish found relationships with proteins involved in muscle contraction, metabolism, heat stress, signalling and other functions [9-12]. The present study used proteins related to beef tenderness and other sensory meat qualities [1-3]. Few reports exist on the relationships between beef colour and other meat quality biomarkers. Many of the proteins identified in other studies have similar biological functions to those used in our study. In agreement with our findings, negative correlations between α-actin and αB-crystallin were found with L^* in pork [13]. Kwasiborski *et al.* [14] found similarly a negative correlation between a Hsp70 isoform (Hsp72 or Hsp70-1A/B) and L^* in pig LT muscle. Zhang *et al.* [15] reported opposite relationships between Hsp90 and L^* and b^* values but not with a^* in pig muscle. Hsp70s are a class of molecular chaperones implicated in the protection of cells from harmful aggregations of denatured proteins during and following various insults such as heat, ischemia and oxidative stress [16]. Hsp70-1A/B, Hsp70-8 and μ-calpaïn were negatively correlated with Enolase 3, a glycolytic enzyme described as a hypoxic stress protein providing protection of cells by increasing anaerobic metabolism [17]. Enolase 3 and MyBP-H were closely associated and positively correlated with L^* and negatively with b^* (Fig. 1a and c). Glycolytic enzymes (e.g., phosphoglucumutase-1,

Table 1. Mean and standard deviation of colour parameters and protein biomarkers abundances of *Longissimus thoracis* muscle of French Blonde d'Aquitaine young bulls.

Variables	Mean	S.D.
Colour		
Lightness (L^*)	36.90	3.24
Redness (a^*)	12.23	1.67
Yellowness (b^*)	15.67	2.57
Protein biomarkers abundances (arbitrary units or %)		
Heat shock proteins		
Hsp27	19.04	5.33
Hsp20	17.39	3.55
αB-crystallin	15.61	4.23
Hsp40	17.40	2.47
Hsp70-1A/B	17.40	3.10
Hsp70-8	17.85	1.64
Hsp-Grp75	17.16	1.90
Metabolism		
Enolase 3	14.51	5.28
LDHB: Lactate dehydrogenase chain B	19.69	3.89
MDH1: Malate dehydrogenase 1	15.71	3.67
Oxidative resistance		
DJ-1: Parkinson disease protein 7	16.13	2.78
Prdx-6: Cis-peroxiredoxin	13.69	1.34
SOD1: Superoxide dismutase Cu/Zn	16.26	1.36
Proteolysis		
μ-calpaïn	16.50	1.99
Structure		
MyBP-H: Myosin Binding protein H	15.18	2.94
CapZ-β: F-actin-capping protein subunit β	15.90	2.33
Actin	19.45	3.58
MyLC-1F: Myosin Light chain 1F	15.74	1.47
MyHC-I: Myosin Heavy chain-I (%)	19.21	3.65
MyHC-IIA: Myosin Heavy chain-IIa (%)	23.85	4.34
MyHC-IIx/b: Myosin Heavy chain-IIx/b (%)	56.94	3.98

GAPDH, *β*-enolase …etc.) were earlier found to be positively correlated with a^* values in beef [10, 11].The glycolytic pathway allows the production of NADH which is an important co-factor influencing metmyoglobin formation [18]. Prdx6 (Fig. 1b), correlated with a* and MDH1, is a bifunctional protein with both glutathione peroxidase and phospholipase A_2 (PLA_2) activities. Prdx6 is further related to apoptosis. This pathway involves phosphatidylserine externalisation (flip-flop mechanism) and phosphatidylcholine internalisation, one of the hallmarks of the onset of apoptosis in *postmortem* muscle. Phosphatidylcholine is a key substrate of PLA_2 and able to neutralise the protons generated by glycolysis. This may explain the strong relationship (r = 0.50; $P < 0.01$) between Prdx6 and MDH1, an enzyme using $NADP^+$ as a cofactor observed in the present study. MDH1 plays pivotal roles in the malate-aspartate shuttle operating

between cytosol and mitochondria. This enzyme is also involved in gluconeogenesis.

In the present study, inducible Hsp70-1A/B and Hsp70-8 were negatively and positively correlated with L^* and b^*, respectively. In accordance, Joseph *et al.* [10] identified three overabundant chaperone proteins related to beef meat colour stability, amongst which a Hsp-1B of 70 kDa. In the present study, Hsp70-1A/B and Hsp70-8 were further positively correlated with μ-calpaïn (r = 0.69 and 0.58; $P < 0.01$). The latter three proteins were also positively correlated (r between 0.61 and 0.75, $P < 0.01$) with MyHC-I (slow oxidative fibres) known to be associated with high levels of Hsp72 [19].

Inducible Hsp70s and μ-calpaïn may influence meat colour interactively. First, *post-mortem*, reactive oxygen species (ROS) damage the endoplasmic reticulum (ER) of the cells liberating Ca^{2+}, which activates in turn, μ-calpaïn. Structural proteins are a major substrate of μ-calpaïn and their characteristics influence meat colour aspects. Second, inducible Hsp70s may limit in this process. Oxidative stress induced by a product of lipid peroxidation, 4-hydroxy-2-nonenal (4-HNE), a α,*β*-unsaturated aldehyde, causes carbonylation of Hsp70 [20], thus inactivating it. It was recently reported that in monkey hippocampal CA1 neurons, carbonylated Hsp70s are key substrates of μ-calpaïn [21]. If this pathway exists also in muscle, this forms of inducible Hsp70, rather than structural proteins, may be hydrolysed by μ-calpaïn [21, 22]. 4-HNE may further contribute to the phenomenon as it may cause disruption of Ca^{2+} homeostasis, membrane and structure damage, and cell death [23]. In support of this hypothesis, 4-HNE was reported to affect meat colour by formation of several adducts with muscle proteins, thus modifying protein functionality [24].

Table 3. Prediction equations (best explanatory models) of colour parameters of *Longissimus thoracis* muscle of Blonde d'Aquitaine young bulls using protein biomarkers.

Dependent variable	Adj-R²	Entered independent variable [1]	Partial R²	Regression coefficient	*P*-value
Lightness (L^*)	0.65***	Enolase 3	0.48	+1.05	0.0001
		Hsp70-Grp75	0.17	–0.51	0.008
Redness (a^*)	0.62**	Hsp40	0.33	+1.22	0.0001
		SOD1	0.12	–0.56	0.004
		Hsp70-8	0.17	–0.57	0.013
Yellowness (b^*)	0.47**	μ-calpain	0.23	+0.64	0.001
		Hsp70-Grp75	0.24	–0.48	0.009

[1] Variables are shown in order of their entrance in the prediction models. Significance of the models: ** < 0.01; *** < 0.001.

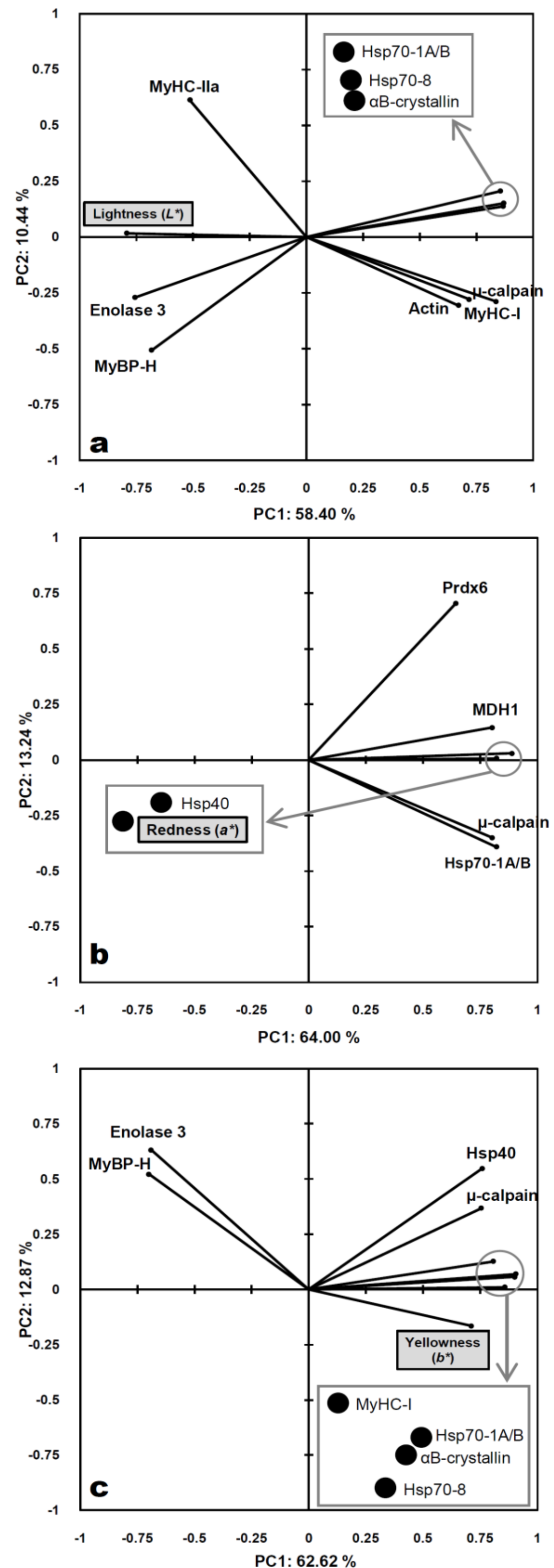

Figure 1. Principal component analysis (PCA) of meat colour traits. **a)** PCA of Lightness (L^*); **b)** PCA of redness (a^*); **c)** PCA of yellowness (b^*) using only the significantly ($P < 0.05$) correlated biomarkers.

These results suggest also that the presence of slow twitch oxidative fibres (type I) has a relatively strong impact on meat colour. First, they contain many mitochondria which generate ROS under hypoxic conditions. In addition, they contain high levels of MyHC-I, inducible Hsp70 and myoglobin, all potential substrates for proteolysis and structural modifications. In conclusion, the interaction between inducible Hsps and μ-calpaïn may defer changes in the structure of pigment and myofibrillar proteins, influencing aspects of meat colour.

The regression models of the L^* a^* b^* are presented in Table 2. The models were significant ($P < 0.01$) and explained between 47 and 65 % of the variability. The models differed according to colour trait, but all show that Hsp proteins play an important role. The models for L^* and b^* included further Enolase 3 and μ-calpain, respectively. Redness (a^*), considered as indicator of meat discoloration, was further related to SOD1 (involved in detoxification of ROS reported to affect meat colour).

IV. CONCLUSION

The present results show that research using proteomics may help to understand the biological mechanisms involved in beef colour development and stability. Colour traits appear to be related to biological pathways that are also involved in tenderness of beef. Hsp70-1A/B, recently described as a good predictor of meat tenderness [3], may thus also play a central role in meat colour traits. Protein biomarkers may be used to predict meat colour and to elucidate the biochemical mechanisms involved.

ACKNOWLEDGEMENTS

This work was part of the EU FP6 Integrated Project ProSafeBeef, contract no. FOODCT–2006–36241. Funding by the European Union is gratefully acknowledged. We thank INRA UERT (Theix) for animal management and slaughter. A grant of the PROFAS B+ program given to M.G. is also gratefully acknowledged.

REFERENCES

[1] N. Guillemin, M. Bonnet, C. Jurie, B. Picard, J Proteomics, 75 (2011) 352-365.

[2] M. Gagaoua, D. Durand, D. Micol, V. Santé-Lhoutellier, C. Terlouw, M.P. Ellies-Oury, A. Boudjellal, J.F. Hocquette, B. Picard, in: 15èmes JSMTV, Viandes & Produits Carnés, Clermont-Ferrand, 2014, pp. 137-138.

[3] B. Picard, M. Gagaoua, D. Micol, I. Cassar-Malek, J.F. Hocquette, C.E. Terlouw, J Agric Food Chem, 62 (2014) 9808-9818.

[4] V. Muchenje, K. Dzama, M. Chimonyo, P.E. Strydom, A. Hugo, J.G. Raats, Food Chemistry, 112 (2009) 279-289.

[5] J. Bouley, C. Chambon, B. Picard, Proteomics, 4 (2004) 1811-1824.

[6] N. Guillemin, B. Meunier, C. Jurie, I. Cassar-Malek, J.F. Hocquette, H. Leveziel, B. Picard, J Physiol Pharmacol, 60 Suppl 3 (2009) 91-97.

[7] B. Picard, C. Barboiron, D. Chadeyron, C. Jurie, Electrophoresis, 32 (2011) 1804-1806.

[8] M. Gil, X. Serra, M. Gispert, M. Angels Oliver, C. Sanudo, B. Panea, J.L. Olleta, M. Campo, M. Olivan, K. Osoro, M.D. Garcia-Cachan, R. Cruz-Sagredo, M. Izquierdo, M. Espejo, M. Martin, J. Piedrafita, Meat Sci, 58 (2001) 181-188.

[9] T. Sayd, M. Morzel, C. Chambon, M. Franck, P. Figwer, C. Larzul, P. Le Roy, G. Monin, P. Cherel, E. Laville, J Agric Food Chem, 54 (2006) 2732-2737.

[10] P. Joseph, S.P. Suman, G. Rentfrow, S. Li, C.M. Beach, J Agric Food Chem, 60 (2012) 3196-3203.

[11] A.C.V.C.S. Canto, S.P. Suman, M.N. Nair, S. Li, G. Rentfrow, C.M. Beach, T.J.P. Silva, T.L. Wheeler, S.D. Shackelford, A. Grayson, R.O. McKeith, D.A. King, Meat Science, 102 (2015) 90-98.

[12] M.A. Desai, P. Joseph, S.P. Suman, J.L. Silva, T. Kim, M.W. Schilling, LWT - Food Science and Technology, 57 (2014) 141-148.

[13] I.H. Hwang, B.Y. Park, J.H. Kim, S.H. Cho, J.M. Lee, Meat Sci, 69 (2005) 79-91.

[14] A. Kwasiborski, T. Sayd, C. Chambon, V. Sante-Lhoutellier, D. Rocha, C. Terlouw, Meat Sci, 80 (2008) 982-996.

[15] M. Zhang, D. Wang, Z. Geng, H. Bian, F. Liu, Y. Zhu, W. Xu, Food Chemistry, 165 (2014) 337-341.

[16] M.P. Mayer, Trends Biochem Sci, 38 (2013) 507-514.

[17] V. Pancholi, CMLS, Cell. Mol. Life Sci., 58 (2001) 902-920.

[18] M. Renerre, R. Labas, Meat Science, 19 (1987) 151-165.

[19] A.R. Tupling, E. Bombardier, R.D. Stewart, C. Vigna, A.E. Aqui, Journal of applied physiology (Bethesda, Md. : 1985), 103 (2007) 2105-2111.

[20] D.L. Carbone, J.A. Doorn, Z. Kiebler, B.P. Sampey, D.R. Petersen, Chem Res Toxicol, 17 (2004) 1459-1467.

[21] S. Sahara, T. Yamashima, Biochemical and biophysical research communications, 393 (2010) 806-811.

[22] E. Nakajima, L.L. David, C. Bystrom, T.R. Shearer, M. Azuma, Investigative ophthalmology & visual science, 47 (2006) 5469-5475.

[23] H. Esterbauer, R.J. Schaur, H. Zollner, Free Radical Biology and Medicine, 11 (1991) 81-128.

[24] S.P. Suman, C. Faustman, S.L. Stamer, D.C. Liebler, J Agric Food Chem, 54 (2006) 3402-3408.