Insights into the background of instrumental chicken breast meat colour as revealed by PLSR

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Relationships among instrumental chicken breast meat colour variables and their associations with physical and biochemical meat properties are highly complex and not fully understood. However, they are important because meat colour influences the purchase decision of consumers. In meat research independent variables are often multicollinear preventing the application of Multiple Linear Regression. To circumvent this problem, relationships among instrumental chicken breast meat colour variables and their associations with pigment traits and physical meat properties were investigated by Principal Component Analysis (PCA) and Partial Least Squares Regression (PLSR) using male birds of an experimental meat-type chicken line (n=40). The instrumental colour profile of breast meat and selected physical meat properties (pH, expressible moisture, cooking loss, shear force) were recorded. Additionally, haem pigment content was assessed by extraction of water soluble myoglobin and acidified acetone extraction of haematin. Pigment traits and physical meat properties explained 41.4 to 59.8% of the variation of colour values in the PLSR models. The important role of haem pigment concentration for the expression of meat colour was confirmed, yielding significant regression coefficients in the PLSR models for most of the colour variables. Besides that, pH traits were major factors determining meat colour. The negative relationship of lightness with redness and colour saturation, respectively, was adequately explicable by the reciprocal effects of haem pigment concentration and the rate and extent of pH fall. In contrast, further research is needed for the allocation of yellowness within the meat colour system. It is hypothesized that chicken breast fillets losing more haeminic pigment through purge will be more yellow due to an increasing impact of carotenoids located in biomembranes.

Keywords: chicken breast fillet; haem pigment; colourimetry; PCA; PLSR.

Introduction

Meat colour of chicken breast fillets is an important quality characteristic influencing the purchase decision of consumers. Because visual appraisals for the determination of meat colour are time-consuming, colour is frequently measured by reflectance colourimetry and reported as CIE (Commission International d'Éclairage) L*a*b* coordinates, sometimes complemented by the polar coordinates chroma and hue angle. Relationships among instrumental colour values and their associations with physical and biochemical meat properties are complex. Hereby, inference can be facilitated using Partial Least Squares Regression (PLSR), which is related to Principal Component Analysis (PCA) and a versatile tool for data modelling and prediction purposes (Wold *et al.*, 2001). In contrast to Multiple Linear Regression prediction variables may be multicollinear, which is a common situation in meat research. In PLSR, a set of orthogonal factors called latent variables is extracted from the predictors (independent variables) to achieve optimum prediction power (Abdi, 2010).

The objective of the present study was to investigate the relationship among instrumentally measured chicken breast meat colour variables and the contribution of pigment traits and physical meat properties to breast meat colour by statistical projection methods.

Materials and methods

In total 40 male birds of an experimental meat-type chicken line, kept by the University of Hohenheim (Stuttgart, Germany) since 2001, were used. The line was established through crossing of the Rhode Island breed with the New Hampshire breed more than 10 generations ago, and segregates for the recessive scaleless (sc) gene causing featherlessness and the naked neck (Na) gene. The birds were heterozygous at the Sc locus, thus normally feathered. Fifteen percent were heterozygous carriers of the naked neck gene. The birds were kept on wood shavings with ad libitum access to feed (week 0-3 starter: 12.6MJ/kg ME, 21.2% CP; week 3-10 grower: 12.5MJ/kg ME, 19.4% CP) and water and each 20 birds were randomly selected and slaughtered at 7 and 10 weeks of age, respectively. Carcases were eviscerated manually and the breast muscle (pectoralis major and minor) was removed. The left pectoralis major muscle was stored at 4°C and used for the determination of meat quality characteristics. From the right pectoralis major muscle 30g slices were harvested at a medial position and stored in plastic tubes at -20°C for the pigment analysis.

The pH of the left pectoralis major muscle was recorded at 20min (±5min) post-mortem (pH_{20}) and at 24h post-mortem (ultimate pH=pH_u) with a pH meter equipped with a glass electrode. At 24h post-mortem the colour profile was determined on the cranial portion of the ventral (adjacent to the skin) and the dorsal (adjacent to the bone) surface of the left breast fillet using a Minolta-branded reflectance colourimeter (model CR-400) with an illuminant C and 2° observer setting. Colour coordinates are reported as L* (lightness), a* (redness) and b* (yellowness) values according to the CIE colour space. From the a* and b* values colour saturation/chroma (C*, $(a^{*2}+b^{*2})^{0.5}$) was derived. Water holding capacity (WHC) of the raw breast fillet was determined at 24h post-mortem using the filter paper press method following van Oeckel (1999). The ratio of meat to expressed moisture (cm^2/cm^2) was obtained, whereas values close to 1 indicate a high WHC. For the determination of cooking loss (CL) at 24h post-mortem samples of the breast fillet were weighed, placed in plastic bags and cooked to an approximate internal temperature of 85°C. Cooking loss was defined as weight loss during cooking relative to initial sample weight. Warner Bratzler shear force (WBSF) was determined on cooked meat samples (2.5mm diameter) at 48h post-mortem. Measurements were conducted perpendicular to fibre orientation with an Instron texture analyzer (model 5565k) equipped with a Warner Bratzler blade at a velocity of 200mm/min.

The extraction of water soluble myoglobin was carried out following the method of Faustman and Phillips (2001) with some modifications. In brief, minced slices (5±0.02g) of the right pectoralis major were mixed with 20ml deionised water and homogenised on ice. After centrifugation the supernatant was filtered by vacuum filtration. Then 2ml of the filtrate were centrifuged at 22,000 x g for 25min at 5°C. The absorbance was read with a spectrophotometer at 730, 582, 557, 525 and 503nm. At a wavelength of 730nm the homogenate is considered to be pigment free (Krzywicki, 1979), thus allowed to correct for turbidity. The concentration of soluble myoglobin in wet tissue was calculated modified after Van Laack *et al.* (1996). The proportions of the myoglobin redox forms metmyoglobin (MetMb), oxymyoglobin (OxyMb), and deoxymyoglobin (DeoMb) were derived from the equations of Tang *et al.* (2004). The determination of total haematin concentration was carried out using acidified 80% acetone solution according to Carpenter and Clark (1995), but without applying a filtration step. Absorbance was read at 640 and 730nm, respectively, to allow correcting for turbidity.

Statistical analyses were conducted using R software version 2.15.1 (R Core Team, 2012). Age groups were compared by Welch's t test. The structure among instrumental colour characteristics was investigated by Principal Component Analysis (PCA). The colour parameters represented the Y variables in the Partial Least Squares Regression (PLSR) models, whereas physical (pH_{20}, pH_u, WHC, CL, WBSF) and biochemical characteristics (myoglobin concentration, haematin concentration, fractions of MetMb, OxyMb, and DeoMb, respectively) were included in the X matrix as predictors. One PLSR model was fitted for each colour trait (measured on the dorsal and ventral surface, respectively). The R package 'pls' by Mevik *et al.* (2011) was used for model fitting. For both, PCA and PLSR, data was centred and scaled. Age groups were pooled for PCA, whereas PLSR was carried out (i) including age as indicator variable, (ii) including live weight as predictor, and (iii) without age and live weight as predictive variables. The number of components (latent variables) for PLSR models was selected using 'leave-one-out' cross-validation based on the root mean squared error of prediction (RMSEP). To perform approximate t tests jack-knife variance estimates were used.

Results and discussion

The loading plot and scores plot for the first two principal components (PC) of the PCA are shown in *Figure 1*. The first PC (vertical line) accounted for 46.2% of the variation, and mainly contrasted lightness and yellowness revealing positive loadings, from redness and chroma, which had negative loadings. On the second PC (horizontal line) explaining 25.4% of variation, lightness assessed on the dorsal surface of the fillet was separated from yellowness, from redness and chroma measured dorsally, and from lightness measured on the ventral side. The negative phenotypic correlation between redness and lightness of chicken breast meat is well established (e. g. Fletcher, 1999). In contrast to Wilkins *et al.* (2000), Nadaf *et al.* (2007) found a positive association between redness and yellowness. Fletcher (1999) reported a weak positive correlation between L* and b* values of broiler breast meat. For fillets pre-sorted according to their L* values, redness of paler fillets was decreased, but yellowness was increased (e. g. Zhuang and Savage, 2010), or remained unaffected (Petracci *et al.*, 2004). Overall, this indicates an inverse relationship between redness and lightness on a given surface, whereas the associations of yellowness with redness and lightness, respectively, are more complex.

Breast fillets from birds slaughtered at an age of 7 weeks were closely related to yellowness and to lightness assessed ventrally on the first two PCs. The ventral surface of fillets of 10 weeks old birds was redder and more intensely coloured, which is in line with the results obtained by Welch's t test (data not shown). Therefore, age has to be considered as confounding factor.

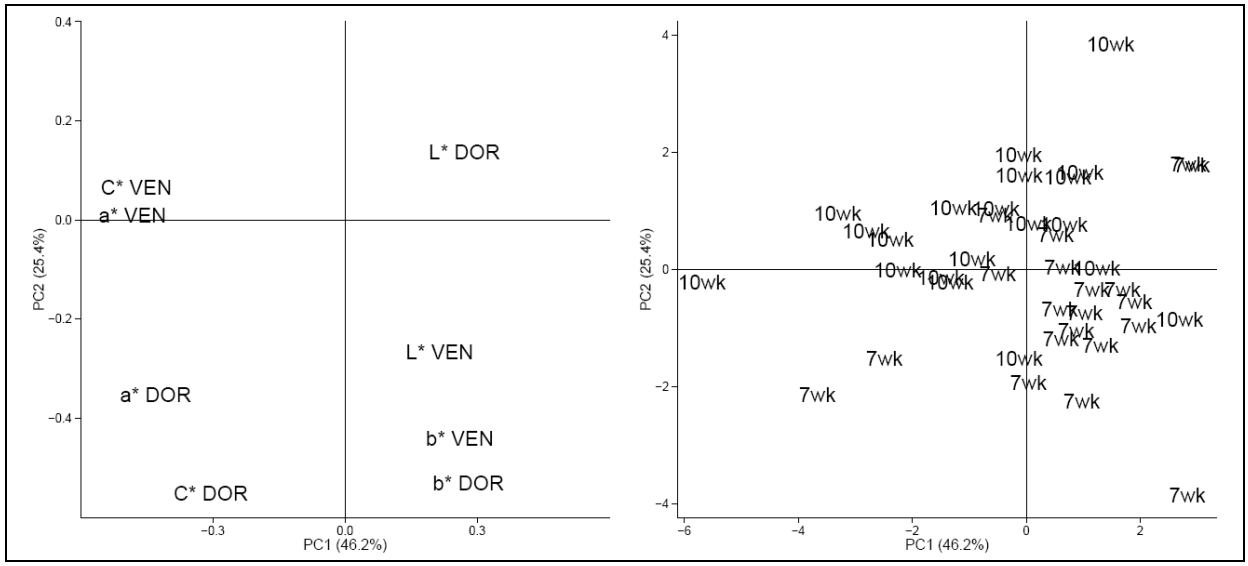

Figure 1 Principal Component Analysis of instrumental colour variables (L*, a*, b*, C*) measured on the dorsal (DOR) and ventral (VEN) surface of breast fillets from chicken slaughtered at 7 (7wk; n=20) and 10 (10wk; n=20) weeks of age, respectively; loadings plot (left) and scores plot (right).

When age or live weight were included as additional indicator variable or predictor, respectively, only minor changes of the standard errors of regression coefficients of the PLSR models were noted. Therefore, results rejecting age as indicator variable and live weight as predictor variable are reported. The PLSR models did not converge for lightness assessed on the ventral side (*Table 1*). Pigment traits and physical meat properties explained 41.4 to 59.8% of the variation in the remaining colour values.

In *Table 1* the regression coefficients for the prediction of colour variables assessed on both surfaces at the optimum number of components are shown. For lightness assessed on the dorsal surface and for yellowness significantly negative regression coefficients for myoglobin content were obtained, whereas myoglobin concentration contributed significantly positive to the prediction of redness and colour saturation. This indicates that the concentration of haem pigments contributes to the negative correlation of lightness with redness and chroma, supporting the findings of Boulianne and King (1995).

Table 1 Variation in the X- and Y variables (colour assessed ventrally [VEN] and dorsally [DOR], respectively) explained by Partial Least Squares Regression (PLSR); PLSR model fit indicated by R^2 and the root mean squared error of prediction (RMSEP); significant regression coefficients ($p<0.05$) are shown in bold

	L*		a*		b*		C*	
x-variable[†]	**VEN**	**DOR**	**VEN**	**DOR**	**VEN**	**DOR**	**VEN**	**DOR**
Components (N)	3		2		2		2	
X-variable (%)	57.4		50.8		49.0		49.9	
Y-variable (%)	n.c.[‡]	59.8	47.0	49.7	49.8	44.5	43.0	41.4
R^2	-	0.34	0.19	0.25	0.31	0.20	0.21	0.17
RMSEP	-	1.579	0.752	0.820	1.068	0.963	0.781	0.872
Regression coefficients								
myoglobin	-	**-0.60**	**0.24**	**0.28**	**-0.34**	**-0.27**	**0.23**	**0.24**
MetMb	-	0.34	-0.05	-0.04	-0.08	-0.06	-0.06	-0.02
OxyMb	-	**-0.35**	0.04	0.03	-0.01	-0.01	0.04	-0.01
DeoMb	-	0.36	-0.01	0.03	-0.04	-0.03	0.01	0.06
haematin	-	0.57	0.13	0.14	-0.20	-0.16	0.14	0.12
pH_{20}	-	0.56	**-0.22**	**-0.27**	**-0.39**	**-0.30**	**-0.25**	**-0.32**
pH_u	-	**-1.04**	**0.16**	**0.20**	**-0.30**	-0.24	0.10	0.12
WHC	-	0.19	0.08	0.08	0.11	0.09	0.11	0.11
CL	-	-0.23	-0.12	-0.14	**0.47**	**0.37**	-0.09	-0.07
WBSF	-	-0.07	**-0.17**	**-0.23**	0.05	-0.04	-0.19	**-0.26**

[†]myoglobin, soluble myoglobin concentration; MetMb, fraction of metmyoglobin; OxyMb, fraction of oxymyoglobin; DeoMb, fraction of deoxymyoglobin; haematin, haematin concentration; pH_{20}, pH measured at 20min post-mortem; pH_u, pH measured at 24h post-mortem; WHC, water holding capacity; CL, cooking loss; WBSF, Warner Bratzler shear force.
[‡]n.c.= not converged

It has to be considered that haemoproteins, i. e. myoglobin and haemoglobin, could not been differentiated by the methods applied. Chicken breast muscle contains low levels of endogenous myoglobin because it is entirely composed of fast twitch glycolytic muscle fibres. Alvarado *et al.* (2007) showed that, even if stunning and bleed-out time were favourable, residual blood remained in the muscle, probably due to a low capillary density. Additionally, it was shown that increased struggling behaviour led to increased a* values of chicken breast meat (Berri *et al.*, 2005), indicating that residual blood contributes to the meat colour of chicken breast fillets. Thus, haemoglobin may have interfered with the determination of water soluble myoglobin.

Besides the concentration of haemoproteins, pH traits played a major role in the determination of meat colour. The regression coefficients from PLSR analysis indicated that a high pH value at 20min after death would decrease both, a* and b* values, whereas an increased ultimate pH would reduce L* and b* values, but elevate a* values. The inverse relationship between lightness and pH_u, though dependent on genetic line, is well established (e. g. Woelfel *et al.*, 2002), and there is also evidence for a positive phenotypic association between redness and ultimate pH (Berri *et al.*, 2005; Fletcher, 1999). In line with the results presented, negative phenotypic correlations between yellowness and ultimate pH were reported (e. g. Nadaf *et al.*, 2007). The decline in pH after cessation of the blood flow at slaughter is caused by the accumulation of lactic acid during rigor development. Declining pH induces alterations in the arrangement of myofilaments, thereby expelling fluids. In consequence, reflectance and light scattering could be increased, causing elevated instrumental lightness values due to a decreased light path through meat (Swatland, 2008). When on the other hand the light path through fillets is extended at limited pH fall, the probability of selective absorption, e. g. through sarcoplasmic proteins, is increased (Swatland, 2008). This model of a reciprocal effect of haem pigment concentration and ultimate pH on meat colour parameters may underlie the first PC in the PCA plot (*Figure 1*). Surprisingly, pH measured at 20min post-mortem, which is an indicator for the rate of pH decline, had an opposite relationship with L* and a* values compared with ultimate pH. However, similar results were reported by Berri *et al.* (2005).

The strong to moderate negative association between lightness and WHC reported by several researchers (e. g. Woelfel *et al.*, 2002) could not be confirmed, because regression coefficients of

indicators related to WHC were close to zero. However, tougher meat was associated with a less red colour, whereas higher moisture losses during cooking were associated with a more yellow colour of the raw product. Thus, effects on functional meat properties generally ascribed to lightness were portioned to associated changes in redness, chroma, and yellowness in the present work. Accordingly, comparatively darker and redder fillets revealed significantly increased pH values, cooking yields, and tenderness (Zhuang and Savage, 2010). In contrast, Galobart and Moran (2004) showed that after freeze-thawing of breast fillets about 10% moisture loss occurred, but both, redness and yellowness, were increased. According to Fletcher (1999), shear force was positively related to lightness, but associated negatively with both, redness and yellowness of broiler breast meat. Similar to the present results, Berri *et al.* (2005) found a negative relationship between yellowness and cook-curing yield for a slow growing chicken line. It appears that the controversial correlations between attributes of meat quality found in literature may partly depend on the genotype-specific expression of trait levels, because it can be assumed that relationships between meat quality characteristics are frequently non-linear (Woelfel *et al.*, 2002).

Basing on the associations with pH_u and haem pigment contents one would expect a close positive relationship between lightness and yellowness as well as an inverse correlation of yellowness with redness and chroma, respectively, but PCA and conflicting results in literature prevent this straightforward interpretation. Recently, it was found that a polymorphism of the β,β-carotene 15,15'-monooxygenase 1 gene (BCMO1) affected the yellowness specifically of breast meat of chicken by altering the content of lutein and zeaxanthin (Jlali *et al.*, 2012; Le Bihan-Duval *et al.*, 2011). An additional effect of the QTL comprising the BCMO1 gene on the a* value (Le Bihan-Duval *et al.*, 2008), could explain concurrent effects of carotenoid levels on yellowness and redness. This could be responsible for the absence of a consistently negative relationship between these colour characteristics. Thus, it is hypothesized that chicken breast fillets, which lose comparatively more haeminic pigment through purge, will have a more yellow colour, but a reduced colour saturation and less red appearance. The impact of carotenoids on meat colour could be increased in this situation, because they are mainly located in biomembranes and therefore possibly less affected by a loss of sarcoplasm.

Conclusions

In conclusion, PCA and PLSR provided insight in the relationships among instrumental colour characteristics and their associations with physical and biochemical chicken breast meat properties. The negative relationship of breast meat lightness with redness and colour saturation, respectively, was adequately explained by the reciprocal effects of haem pigment content, the derivatives of haemoproteins, and the effects of rate and extent of pH fall. This indicates that peri-slaughter conditions such as stress and bleed-out efficiency are important in determining the appearance of breast meat. However, also ante-mortem factors such as genotype, age and body weight have to be considered with respect to possible differences in endogenous pigment content or stress susceptibility. Further research is needed for the allocation of yellowness within the meat colour system.

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