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Visible spectroscopy as a tool for the assessment of storage conditions of fresh pork packaged in modified atmosphere

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ABSTRACT

The storage conditions of fresh meat are known to impact its colour and microbial shelf life. In the present study, visible spectroscopy was evaluated as a method to assess meat storage conditions and its optimisation. Fresh pork steaks (*longissimus thoracis et lumborum* and *semimembranosus*) were placed in modified atmosphere packaging using gas mixtures containing 0, 40, 50, and 80% oxygen, and stored with or without light for up to 9 days. Principal component analysis of visible reflectance spectra (400–700 nm) showed that the colour of the different meat cuts was affected by presence of oxygen, illumination, and storage time. Differences in the oxygen levels did not contribute to the observed variance. Predictive models based on partial least squares regression–discriminant analysis exhibited high potency in the classification of the storage parameters of meat cuts packaged in modified atmosphere. The study demonstrates the applicability of visible spectroscopy as a tool to assess the storage conditions of meat cuts packaged in modified atmosphere.

1. Introduction

Colour is the major descriptive characteristic of meat and a principal factor in consumer purchasing decisions (Mancini & Hunt, 2005). In an oxygen-rich (70–80%) environment, oxygen (O_2) molecules diffuse in the meat surface layer, resulting in the formation of the bright red oxymyoglobin on the product surface (Mancini, Hunt, & Kropf, 2003), which is visually-appealing (Kropf, 1980). Therefore, high-oxygen modified atmosphere packaging (MAP) is common in the retail packaging of fresh meat, despite documented adverse effects on sensory quality aspects, caused by oxidative modification of the structural proteins (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007) and of lipids (Cayuela, Gil, Bañón, & Garrido, 2004). The extent of this deterioration has been shown to be dependent on O_2 level in the MAP package and on the fresh meat product type (Bao & Ertbjerg, 2015; Spanos, Tørngren, Christensen, & Baron, 2015). In addition, meat cuts are originating from different muscles that are known to exhibit different physiological characteristics (such as myoglobin content), and this may be a determining factor in the stability of meat in MAP during storage. Meat colour is tightly related to meat oxidative status and the meat pigment myoglobin, which has been showed to promote lipid and protein oxidation. Therefore, the surface colour characteristics of an individual meat cut could yield information regarding fresh meat quality and relate to its storage conditions. The use of visible (Vis) reflectance spectroscopy (400–700 nm), could potentially be implemented as a fast track method for the assessment and prediction of the impact of storage conditions on the colour of retail-packed fresh meat. Due to the rapid, non-destructive nature of the method, this could also allow for muscle-specific optimisation of storage conditions and individual adjustment of shelf life, resulting in a more consistent product quality at purchase.

Spectroscopic techniques, such as Vis spectroscopy, result in datasets that contain a large number of recorded variables. As these multivariate datasets can be difficult to interpret, meat colour has been commonly assayed through the use of colour spaces, like the CIE L*a*b* and Hunter systems, or by using the intensity of specific myoglobin maxima in the Vis spectra (Krzywicki, 1979). This simplifies the data analysis, but useful information may be lost from compression or discarding of data inherent to these methods. Multivariate data analysis techniques such as Principal Component Analysis (PCA) and Partial Least Squares Regression (PLS) allow the utilisation of a vast number of variables and can be a powerful tool in the processing of spectroscopic data.

Previous studies have investigated the use of multivariate data analysis in the identification and prediction of meat quality parameters. Jun et al. (2007) classified pork into different quality categories by using reflectance in the Vis/near infrared (NIR) range. Liu et al. (2003) were able to predict the colour and tenderness of beef using Vis/NIR, while Wu et al. (2014) demonstrated the usefulness of PCA of NIR spectra in

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tandem with an unsupervised learning algorithm in the classification of pork sample freshness. More recently, Vis/NIR hyperspectral imaging alongside partial least squares regression–discriminant analysis (PLS-DA) was successfully used to differentiate thawed from fresh pork (Ma et al., 2015) and to detect adulteration of pork with meat of a different species (Ropodi, Pavlidis, Mohareb, Panagou, & Nychas, 2015). However, the use of Vis spectroscopy in combination with multivariate analysis for the assessment of storage conditions and history of pork packed in MAP has not been previously investigated.

Therefore, the aim of the present study was to examine whether storage conditions of fresh meat cuts packed in MAP with different gas composition with or without light exposure could be differentiated using Vis spectroscopy, and multivariate data analysis. Furthermore, our investigation also aims at revealing whether Vis reflectance spectroscopy in combination with multivariate data analysis could be used as a tool to predict the storage stability and colour development of fresh meat packed in MAP. Development of such mathematical models could enable on-line assessment of shelf life and early detection of reduced colour stability, and hence allow optimisation of the shelf life of fresh pork cuts.

2. Materials and methods

2.1. Fresh meat cuts

Fresh meat cuts consisted of two different retail cuts of pork (chops and schnitzels) originating respectively from the *Longissimus thoracis et lumborum* (LTL) and from the *Semimembranosus* (SM) muscles. A total of 36 female pigs with a weight between 79 and 87 kg were selected after slaughter at a Danish slaughter house. The day after slaughter, pH_{24} was recorded on the left loins, the carcasses were cut, deboned and trimmed before crust freezing at −34 °C for approximately 4 min in an industrial impingement freezer. For LTL, 36 loins (left and right) from 18 pigs were excised and sliced into 2-cm thick chops using an industrial slicer, subsequently packed in polystyrene boxes and transported at 0 °C to the packaging lab before MAP and storage. For SM, the left topsides from18 pigs were used and were sliced into 1-cm thick chops using an industrial slicer, packed in polystyrene boxes and processed as previously mentioned for LTL.

2.2. Packaging and storage

Upon arrival to the packaging lab, LTL and SM were placed with two slices per tray in M71-51A Black MAPET trays (oxygen permeability: 0.63 cm^3/tray/24 h/atm) (Færch Plast Holstebro, Denmark), filled with one of the five gas mixtures (Table 1) and sealed using TOPSEAL.PET.MAP.P.B.AF.62 film (oxygen permeability: 0.3 cm^3/m^2/24 h/atm) (Færch Plast Holstebro, Denmark). Slices from six different animals were used for each of the 3 storage durations, and the packaged samples were stored at 5 °C for 2, 6, and 9 days. For each of the animals, slices originating from single SM or LTL muscles were distributed between the 5 gas compositions used on the study. The totality of LTL slices (n = 90) and SM slices (n = 90) were illuminated under 1200 lx fluorescent light (Certus T5, Riegens, Denmark) for twelve hours per day while an additional set of LTL was kept in the dark for 4 of the above mentioned animals (n = 60).

Table 1
Gas composition of MAP pork LTL and SM samples.

Gas	Anoxic	Lower/intermediate	Intermediate	High CO_2	High oxygen
O_2	0%	40%	50%	50%	80%
CO_2	20%	20%	20%	40%	20%
N_2	80%	40%	30%	10%	-

2.3. Colour measurements

Colour coordinates (L*, a*, and b*) of the CIE 1976 colour space, as well as reflectance spectra in the Vis range (400–700 nm) at a resolution of 10 nm were obtained from all samples using a Konica Minolta Spectrophotometer CM-600d (Minolta Co. Ltd., Japan), at an 8 mm aperture and 10° standard observer. Illuminant D65 was used as a light source with a correlated colour temperature of 6504 Kelvin. All measurements were performed in triplicates at different locations on the surface of the meat samples, avoiding visible fat tissue, and all replicates are retained as individual objects in the dataset. Obtained values for each replicate were the average of 5 consecutive measurements taken at the same location. Measurements were obtained immediately after unsealing of each package and 30 min after exposure to atmospheric air at 2 °C, allowing the surface to bloom. This resulted in a total of 900 objects for LTL and 540 objects for SM.

2.4. Data analysis of colour values

Statistical analysis of the L*, a*, and b* values was performed separately for LTL and SM using OriginPro (version 9.0.0, OriginLab Corporation, USA). As the interaction term of the two factors (gas composition and storage time) was not significant at the $P < 0.05$ level, means were compared between groups using one-way analysis of variance for each of the examined factors.

2.5. Vis spectral data pre-processing and exploratory analysis

Raw spectra were pre-processed using a standard normal variate transformation followed by mean centring, to remove the multiplicative interferences from non-specific scatter while preserving the autocorrelation between variables. Exploratory PCA models were constructed on the transformed spectral data without validation. All preprocessing was performed through the routines available in the PLS_toolbox (Version 7.9, Eigenvector Technologies, Manson, USA), used in MatLab (Version 2013a, MathWorks).

2.6. Predictive model (PLS-DA) formulation

Unless otherwise stated, samples packed without O_2 were removed from the dataset and the remaining LTL (n = 720 objects) and SM (n = 432 objects) samples were used for the formulation and testing of the PLS-DA models. Objects corresponding to half of the animals in each set were used to form the calibration sets for LTL (n = 432) and SM (n = 216). For LTL, the calibration set was larger than the validation set as additional samples stored in the dark were included. The remainder of the objects per sample group were used as independent validation sets for LTL (n = 288) and SM (n = 216). Data forming the Y matrix in the PLS-DA calibration were autoscaled. The resulting PLS-DA models (Table 2) were validated with a random subsets routine (100 data splits, 20 iterations) available in the PLS_toolbox. The number of components in the calibration models was selected on the basis of the Root Mean Square Error of Calibration (RMSEC), the Cross Validation Coefficient of Determination (R^2CV) and the Root Mean Square Error of Cross Validation (RMSECV), as well as on the total variance explained, based on an iterative approach after each outlier removal. The efficiency of the PLS-DA classification models was summarised by percentage of cases correctly classified (CCR), per class.

3. Results and discussion

3.1. Characterisation of meat colour

The use of the L*, a*, and b* values is common in the reporting of instrumental measurements of colour (Tapp, Yancey, & Apple, 2011).The