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Potential use of visible reflectance spectra to predict lipid oxidation of rabbit meat

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ABSTRACT

This study was performed to explore the feasibility of using the visible spectral region (360–740 nm) for the rapid and non-destructive prediction of lipid oxidation in rabbit meat stored at 4 ± 1 °C for 1, 6 and 12 days.

The analyses were performed on vacuum packed meat stored in darkness at 4 ± 1 °C on day 1, 6 and 12. Spectra were analysed through multivariate regression (PLSR) calibration model, using the full spectral region between the pre-treated spectra by second derivatives and the reference TBARS values.

The high coefficient of determination ($R^2 = 0.87$) between the predicted and measured TBARS values as well as the high values of the root-mean-square errors of calibration and prediction (RMSEC = 0.478 and RMSEP = 0.532) suggested the usefulness of visible spectroscopy in predicting meat oxidation.

Furthermore, the suitability of five wavelengths (380, 420, 440, 580 and 600 nm), selected by PLSR, to discriminate meat samples according to storage times, was also investigated by discriminant analysis performed on a new data set, composed of the second derivative spectral data, measured on meat samples (n = 900), which were collected from local supermarkets at 3 different storage times (1, 3 and 6 days). Overall accuracy was 82%, thus corroborating the feasibility of using visible spectroscopy to assess the qualitative decay in rabbit meat.

1. Introduction

The production and consumption of rabbit meat is ubiquitous in certain Mediterranean countries, especially in regions where it is associated with cultural, traditional and religious reasons (Dalle Zotte, 2002). Rabbit meat is very lean and easily digestible, and lipids contain a high proportion of polyunsaturated fatty acids (Lazzaroni et al., 2009).

The lipids of rabbit meat can suffer alterations during refrigerated storage, owing to lipolysis and oxidation, resulting in quality deterioration characterised by off-odours, off-flavours, colour and texture defects, loss of nutritional value (Alasnier et al., 2000) and the possible production of toxic substances (Monahan, 2000).

Colour is one of the primary meat sensory factors, and its variation during storage can be characterised by visible absorption (Liu and Chen, 2001). Many authors have shown that lipid oxidation and pigment oxidation are closely related in beef (Faustman et al., 2010) and that lipid oxidation is a promoter of myoglobin oxidation and both reactions can influence each other. The aldehyde products of lipid oxidation initiate conformational changes in myoglobin, causing increased heme oxidation and browning (Alderton et al., 2003).

Spectroscopy, in combination with chemometrics, has been shown to be an outstanding tool for the rapid analysis of food, and it can be utilised in research and the food industry (Andrés et al., 2008). Non-invasive spectroscopic techniques can be used to measure simultaneously and in situ the individual food components in the food matrix, and chemometrics can be used to effectively extract quantitative information and the underlying qualitative features (the latent structures) from the multivariate

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and covariate spectral data (Prieto et al., 2009). The use of the optical properties of meat for quality control has been discussed for several decades (Qiao et al., 2007). Many studies have developed models to predict meat pH (Monin, 1998), drip loss (Geesink et al., 2003), colour (Leroy et al., 2004) and tenderness (Rødbotten et al., 2000), using the 400–1100-nm wavelength range with the partial least squares (PLSR) method. Although these studies indicate that Vis/NIR spectroscopy is feasible and promising for applications in the quality control of meat products, the results, particularly for the prediction of meat oxidation, have proven to be inconsistent.

Existing studies (Cifuni et al., 2014) have focused on evaluating the influence of hunting methods on the colour and lipid-oxidation state of meat from ungulates and demonstrated that visible spectroscopy can be useful for predicting meat oxidation.

The spectrophotometric thiobarbituric acid reactive substance (TBARS) method is the most frequently used test for malondialdehyde (MDA) quantification, especially in muscle tissues, as a marker of lipid peroxidation. However, the TBARS method has been criticised as lacking specificity and adequate sensitivity towards malondialdehyde. High-performance liquid and gas chromatographic methods offer better specificity and sensitivity for malondialdehyde detection. However, most of these techniques are destructive, tedious and time-consuming, making them unsuitable for on-line applications (Liu et al., 2004a; Prieto et al., 2008). Rapid, sensitive, economical and reliable methods for the detection of meat oxidation during refrigerated storage are crucial for product quality control (Cen and He, 2007; Meza-Márquez et al., 2010).

This study was performed to explore the feasibility of using the visible spectral region (360–740 nm) for the rapid and non-destructive prediction of lipid oxidation in rabbit meat stored at 4 ± 1 °C for different lengths of time, until quality decay.

2. Materials and methods

2.1. Collection of meat samples

The trial was performed on a commercial rabbit farm located in northern Italy, using Martini rabbits breed.

At weaning (39 days), the rabbits were housed in collective cages according to conventional rearing systems. The collective cages were located on the rabbit farm and were supplied by a forced ventilation system (temperature, 18 ± 3 °C; relative humidity, 60–65%; and a photoperiod of a 16-h light phase). Each cage housed four animals, and the density was 14 rabbits/m^2. The experimental groups received ad libitum a commercial pelleted feed (protein, 17.0%; fat, 3.3%; and digestible energy 10.8 MJ/kg) until 90 days of age, when they were electrically stunned and slaughtered at an abattoir in accordance to the current E. U. regulations (E.C., 2009). In this trial, 50 randomly selected animals, with a slaughter weight of 2.528 ± 0.193 kg and a carcass weight of 1.520 ± 0.150 kg were used. The semimembranosus muscle was separated (approximately 120 g of meat) from both thighs and used for colour evaluation. Then, the apical portion of each muscle was separated and used as a control (day 1). The other two portions of muscle were vacuum packed and stored in the dark at 4 °C for 6 and 12 days, the latter has been considered to be possible end of shelf life. 50 samples were used for each storage time case.

Further to assessing the usefulness of selected wavelengths, by PLSR, to discriminate meat samples according to storage times, the new data set were obtained by 900 samples of semimembranosus muscle, separated from thigh rabbits, belonging to commercial carcasses, collected from local supermarkets. The semimembranosus muscle samples, covered with oxygen permeable film and stored in the dark at 4 ± 1 °C for 1, 3 and 6 days were subjected to visible spectrometric analysis.

2.2. Proximate composition

The dry matter and proximate composition of meat samples, stored for 1 day, was determined in duplicate on the remaining thigh muscles, finely ground, using AOAC standard procedures (1995). The protein determination was done using the Kjeldahl method, multiplying nitrogen content by 6.25. The fat content was determined on hydrolysed meat samples by HCl 3N, and extracted by Soxhlet apparatus, using anhydrous dietylether as solvent. The moisture was quantified by oven drying 10–15 g of samples at 105 °C overnight. The ash content was determined by combustion in a muffle furnace at 550 ± 1 °C.

2.3. Reflectance spectra

The visual reflectance spectra between 360 and 740 nm of the raw meat were determined by using a MINOLTA CM 2600 D spectrophotometer with a D65 source after 1 h of oxygen exposure. Six spectra per sample with a resolution of 10 nm were recorded. From the obtained spectra the second derivative spectra were calculated by Unscrabler software (CAMO) using the Savitzky-Golay derivative function with 2 smoothing points. Furthermore, the integral values of the spectra were calculated in the spectral ranges of 440–540 nm, 540–580 nm and 580–740 nm, i.e. the spectral regions showing the highest reflectance values.

2.4. Lipid oxidation

Lipid oxidation was measured by TBARS test according to Bergamo et al. (1998). The samples were homogenised in water supplemented with butylated hydroxytoluene. The proteins were precipitated with 10% ice-cold trichloroacetic acid and removed by centrifugation. The supernatant was incubated at 90 °C for 30 min in a 0.28% thiobarbituric acid (TBA) mixture. The MDA–TBA adduct was fractionated by reverse-phase HPLC and detected by fluorescence (λEX = 515 nm; λEM = 543 nm). Elution was performed at a 1-mL/min flow rate with a mixture of acetonitrile and sodium phosphate at pH 7 (15:85 v/v). The MDA–TBA peak was identified by the elution profile of authentic standard. A calibration curve was performed using TEP (1,1,3,3-tetraethoxypropane) solutions of concentrations varying from 0.007 to 1.25 mg/mL. The TBARS concentration was expressed as mg of MDA/kg of meat and triplicate analyses were performed for all samples. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.00042 and 0.0014 mg/mL, respectively.

2.5. Statistical analysis

Statistical analysis was performed by the GLM procedure of SAS (2012) with storage time as the factor. Pearson's coefficient of correlation was calculated to determine the dependence between TBARS values and the second derivative data of the visible spectra (370 nm–730 nm). The second derivatives of spectral data points were processed using a partial least squares regression (PLSR) multivariate analysis to predict the TBARS from the reflectance information (Martens and Martens, 2001) using Unscrambler software (CAMO software, 2011). PLSR analysis was performed on all samples without separating them into experimental groups.

To optimise the accuracy of the calibration and to identify the outliers, the detection of anomalous spectra in the calibration dataset was accomplished using the Mahalanobis distance to the centre of the population (Williams and Norris, 2001). The outliers were removed from the calibration dataset and thus the final dataset available for investigation included 128 samples. The prediction models were processed using cross-validation by dividing