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Predicting the Total Volatile Basic Nitrogen (TVB-N) Content of Wet-aged Beef and Lamb Meat Using Near Infrared Spectroscopy

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Abstract

This study aimed to validate the use of near-infrared spectroscopy (NIRS) to predict total volatile basic nitrogen (TVB-N) concentration for wet-aged samples of beef and lamb M. *longissimus lumborum* samples and to determine if a combined species calibration model could be developed. Partial least squares (PLS) regression models were developed for species-specific (beef and lamb) and combined-species datasets. Beef samples exhibited a TVB-N range of 4.0-17.0 mg/100 g, while lamb samples ranged from 5.4-7.2 mg/100 g. The combined-species model demonstrated strong predictive performance ($R^2 = 68.0$), although residual prediction deviation values indicated that a larger and more diverse dataset would substantially improve model robustness and precision. Nonetheless, these preliminary findings do support the application of NIRS to rapidly and non-destructively predict TVB-N concentrations, using freeze-dried and ground samples of red meat.

Keywords

Near infrared spectroscopy (NIRS); total volatile basic nitrogen (TVB-N); partial least square (PLS) regression; freeze-dried samples; red meat; freshness; predictive calibration models

1. Introduction

Meat quality and consumer acceptability are determined by a complex interplay of organoleptic and physicochemical traits, including nutritional composition, colour, flavour, texture, and microbial safety. While ‘freshness’ is often used to describe meat of satisfactory quality that adheres to microbial safety guidelines, its direct measurement remains a challenge. Consequently, total volatile basic nitrogen (TVB-N) has emerged as a candidate universal biomarker for meat freshness. TVB-N includes trimethylamine (TMA), dimethylamine (DMA), and formaldehyde via reduction of trimethylamine N-oxide (TMAO), volatile nitrogenous compounds that accumulate as microbes and endogenous enzymes degrade proteins and amines [1]. TVB-N concentrations will typically increase as meat is stored, meaning it may be related to microbial growth and the sensorial decline associated with spoilage [2, 3].

Within this context, TVB-N has been used to estimate the freshness of fish [4] and, more recently, other meat products such as pork, chicken, and [5-7]. Thresholds have been proposed for the point at which TVB-N indicates that meat is spoiled [8-10], with some recommendations for beef or red meat products proposing spoilage to occur when TVB-N > 15 mg/100 g [11, 2], however more recent research has found that acceptable consumer thresholds of freshness may be significantly lower for beef, closer to 5.1 mg/100g [12]. Irrespective, these mean that the quantification of TVB-N must be accurate and precise for red meat products.

Historically, the estimation of TVB-N content has primarily been determined using well-established wet chemistry techniques developed for analysing levels of TVB-N in fish, with many of these techniques having been adapted from the pioneering technique developed by Conway [13] and involving the extraction of a sample in solution followed by distillation and titration on a distillation apparatus [14-16]. While reliable, these methods are labour-intensive, require specialist equipment, and are destructive to the sample. Advances in rapid, non-destructive technologies, such as Near-infrared Spectroscopy (NIRS) and Hyper spectral Imaging (HSI), have enabled researchers to rapidly and accurately predict many meat quality traits such as intramuscular fat (IMF) content [17-22], with HSI often favoured, particularly in the seafood industry due to its ability to extract spectral data as well as both temporal and spatial features such as colour and texture at the pixel level, providing a more comprehensive analysis [23-25]. Despite this limitation, NIRS remains the more widely adopted technology for food quality and freshness predictions, particularly in meat, likely due to the challenges that free moisture presents for HSI [25]. However, little research has been done evaluating the suitability of using freeze-dried ground reference samples for predicting TVB-N in red meat. The purpose of this study is twofold: first, to assess the predictive performance of NIRS-based models for TVB-N across a broad range of samples representing different wet-aging periods and microbial spoilage levels; and secondly, to compare species-specific calibrations (beef and sheep) with a combined-species calibration to determine which, if any, approach yielded the more robust calibration model.

2. Materials and Methods

The samples used in this study were sourced from two studies by Holman et al. [12]. Briefly, in experiment 1, 24 beef striploins (*longissimus lumborum*) were selected randomly from a commercial abattoir. At approximately 48-hours *post-mortem*, each striploin was divided into six equal portions and randomly assigned to six ageing periods (0, 3, 5, 8, 11, and 14 weeks). Individual portions were held *in vacuo* at ~ 0.5 °C until the completion of their assigned ageing period and thereafter held frozen at -20 ºC until analysis.

In the second experiment, 72 lamb loins (*longissimus lumborum*) were collected from trial lambs slaughtered at a commercial abattoir. At approximately 48-hours *post-mortem*, each strip was sampled and again held *in vacuo* at ~ 0.5 °C for 5 or 56 days, then held frozen at -20 ºC until analysis. During the sample collection for both experiments, and at the same time as TVB-N sampling, approximately 25.0 g of chopped lean meat was also taken from each loin and freeze-dried at -50 °C (ScanVac CoolSafe™, LaboGene Ltd., Lynge, DEN) and ground using a sample mill (model 1095, Knifetech™, FOSS Pacific Ltd., New South Wales, AUS) to generate a homogenous sample for subsequent NIR scanning and calibration development. Similarly, lean tissue samples of ~ 10 g were prepared, devoid of connective tissue and fatty deposits, vacuum packaged, and held frozen at -20 ºC for TVB-N analysis.

TVB-N content was determined using the method described by Holman et al. [12] based on the steam distillation method GB 5009.228-2016 [11]. This involved the homogenisation of ~ 10 g of thawed sample in 100 mL of distilled water using a blender (Ultra 1200, Nutribullet, AUS). The homogenate was placed in a sealed jar and kept at room temperature for 30 min, shaking every 10 min. Samples were then filtered through Whatman #1 paper and refrigerated (2-3°C) overnight. Analysis was performed using an automated Kjeldahl distillation unit (Kjeltec 8400, FOSS, (Kjeltec 8400, FOSS, Denmark). Specifically, a 10ml aliquot of the filtrate was transferred to a glass distillation tube, followed by 10 mL of magnesium oxide solution (10 g/L), and this solution was exposed to 5 min of steam distillation. The resulting distillate was condensed into a receiving flask containing boric acid (20 g/L) and bromocresol green/methyl red, mixed indicator (Sigma Aldrich, Australia). Finally, the solution was back titrated with 0.01 M hydrochloric acid solution (ChemSupply, Australia), and the TVB-N was calculated as mg/100 g raw meat (as-is).

3. Spectral Analysis

Freeze-dried and ground samples were packed into glass NIR vials (2 × 4 cm) to ~ 75% of their volume and tamped to achieve uniform density. Vials were stored at −18 °C until all proximate analyses were completed. Prior to spectral acquisition, vials were allowed to equilibrate at room temperature for ~ 2 h.

NIR spectra were collected using a multipurpose NIR analyser (MPA II, Bruker, USA), across the 8500-3700 cm^{-1} (1176-2703 nm) range, and at a spectral resolution of 8 cm^{-1}. This range selection was based on Bae et al. [26] recommendations for optimal TVB-N calibration**.** For each sample, 32 scans were performed and averaged to produce a single spectrum per sample. Data pre-processing involved Spectral mean centring, followed by a 17-point smoothing function to enhance the signal-to-noise ratio.

4. Data Analysis

Spectral datasets were partitioned into three groups: sheep meat, beef, and combined beef and sheep meat. Group datasets were imported into OPUS (Bruker Optik, OPUS v7.5, 2014) and were randomly partitioned into calibration and independent validation subsets based on their principal component analysis (PCA) scores. Specifically, 20-30% of samples per group were reserved for the independent validation (prediction) set, with the remaining samples comprising the calibration (cross-validation) set (Table 2). Calibration models for TVB-N were developed separately for sheep, beef, and combined species. Partial least squares (PLS) regression was performed in OPUS using the calibration sets and the averaged spectral scans as input, with different mathematical pre-treatments evaluated during model optimisation. First-derivative plus multiplicative scatter correction (MSC) was selected as the optimal pre-treatment; the combination was then applied to the final calibration models. Model performance was assessed by evaluating the root mean square error of cross-validation (RMSECV) and the coefficient of determination (R^2), and then using the independent validation set and quantified by the root mean squared error of prediction (RMSEP).

5. Results and Discussion

Many studies have examined the use of NIRS for predicting TVB-N levels in fish [27], poultry [28], pork [29, 23], and other meats [2]. However, comparatively few investigations have focused on beef and lamb, and none have utilised freeze-dried and ground samples for analysis and calibration development. Of the limited studies using fresh beef [30, 31], calibration models showed potential, achieving strong correlation coefficients even though their data sets were relatively small.

Table 1 summarises the TVB-N values included in the current study. Lamb samples exhibited a narrow range of TVB-N, between 5.4-7.2 mg/100g, whereas beef samples ranged from 4.0-17.0 mg/100g. These values align with those reported by Kim et al. [31] and Leng et al. [32], although Leng et al. observed a slightly broader range for beef samples, with TVB-N content between 12.1 and 22.0 mg/100g. The range of TVB-N contents for the combined calibration ranged from 4.0 to 17.0 mg/100g, covering the range expected for both fresh and aged samples of red meat. While extended ageing periods, up to 14 weeks post-mortem, were expected to generate higher TVB-N concentrations, Holman et al. [12] observed that few actually exceeded the threshold value of TVB-N > 15 mg/100 g [11, 2].

Table 1. Summary of total volatile basic nitrogen (TVB-N) data (mg/100g) for the beef and lamb *longissimus lumborum* muscle samples

Species	Muscle	*n*	Mean	Range	Standard Deviation
Lamb[1]	*M. longissimus lumborum*	72	6.0	5.36-7.23	0.31
Beef	*M. longissimus lumborum*	144	5.95	3.96-17.0	1.65

Predictive calibration models were developed using PLS regression applied to pre-processed spectral data. Table 2 reports sample numbers, coefficient of determination (R^2), root mean square error of cross-validation (RMSECV), and root mean square error of prediction (RMSEP) for both the cross-validation and independent prediction data sets, while a plot of the predicted versus true TVB-N from the combined lamb and beef samples can be seen in Figure 1. Spectral outliers identified by the OPUS software were excluded from the analysis.

Table 2. Accuracy of calibrations developed to predict total volatile basic nitrogen (TVB-N) concentration of freeze-dried ground beef and sheep meat

	Cross validation				Prediction			
	n	RMSECV	R_{cv}^2	RPDc	*n*	RMSEP	R_p^2	*RPDp*
TVB-N (mg/100g)								
Lamb meat	54	0.26	9.81	1.04	17	0.26	19.93	1.07
Beef	103	0.44	78.0	2.13	33	0.55	81.0	2.5
Combined	151	0.42	68.0	1.77	52	0.49	75.0	2.0

Notes. RMSECV: Root Mean Squared Error of Cross-Validation, R_{cv}^2: Coefficient of determination in cross-validation, RMSEP: Root Mean Squared Error of Prediction, R_p^2: Coefficient of determination in prediction; RPDc: residual prediction deviation in cross validation; RPDp: residual prediction deviation in prediction.

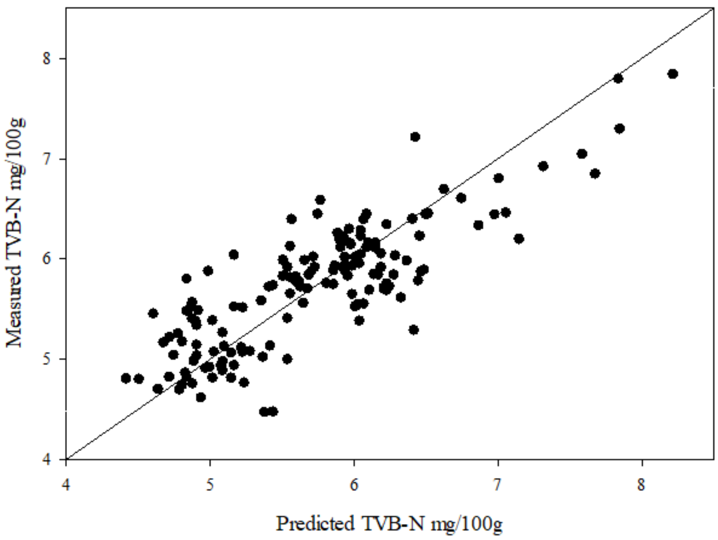

Figure 1. Relationship between laboratory-determined TVB-N (mg/100g) and TVB-N predicted by NIRS on freeze-dried beef and lamb.

The resulting PLS models for beef and combined beef/sheep meat demonstrated strong predictive performance, with high R^2 values and low RMSECV and RMSEP, indicating good predictive accuracy and robustness (Table 2). This aligns with the study by Leng et al. [31], who found PLS models to be superior to Support Vector Regression (SVR) analysis. In contrast, the individual calibration for lamb meat showed significantly lower accuracy compared to beef data (R_{cv}^2 = 9.81, RMSECV = 0.26) compared to beef (R_{cv}^2 = 78.0, RMSECV = 0.42). This reduced performance is likely attributable to the smaller number of samples and the substantially limited TVB-N range in the lamb meat data set used – TVB-N concentrations for these samples ranged from 5.4 to 7.2 mg/100 g. Improving the precision of the lamb-only calibration would require a broader range of TVB-N and greater spectral diversity.

Residual prediction deviation (RPD) for all models was found to be below the threshold considered to be acceptable for analytical purposes. Kamruzzaman et al. [33] recommended that the RPD > 3 is necessary for practical use. The inclusion of a greater number of both beef and lamb samples, particularly those with TVB-N concentrations > 6.5 mg/100 g, may help improve model robustness and should be explored in future research.

6. Conclusion

This study demonstrates the potential of NIRS to rapidly and accurately predict TVB-N concentrations using freeze-dried and ground samples of beef and lamb meat. The developed PLS calibration models for beef and for the combined beef/lamb data showed strong predictive performance, suggesting that NIRS may be suitable for high-throughput proximate analysis of these sample types. Further refinement of the models, through the inclusion of greater sample diversity and higher TVB-N concentrations, would be beneficial, particularly before the adoption of any models in a research or commercial setting.

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Compliance with Ethical Standards

Conflict of Interest: None.
Ethical Approval: This article does not contain any studies with human participants or animals performed by any author
Informed Consent: Not applicable.

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