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Identifying animal species in NIR hyperspectral images of processed animal proteins (PAPs): Comparison of multivariate techniques

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ABSTRACT

The use of PAPs in animal feed has several advantages over other feed ingredients, but requires rigorous and accurate control mechanisms that ensure the absence of ruminant meal. In order to differentiate between animal species while simultaneously offering the capacity to inspect PAPs in large volumes, a hyperspectral imaging (HSI) system operating in the NIR spectral range is proposed. This study investigates the sensitivity, specificity and other parameters with which HSI can discriminate between different animal species (ruminants, swine and poultry), making use of various classification methods. Diffuse reflectance spectra were acquired from 125 rendered meal samples in the 1000–1700 nm wavelength range; measured PAPs included particles of scale, hair, feather, blood, grease, skin, muscle and bone from both ruminant and non-ruminant animals, obtained in a rendering plant. Various classification methods were then applied to the dataset to determine the accuracy with which different animal species could be discriminated from each other. Support Vector Machine classification performed best in discriminating between animal species, with a sensitivity and specificity of around 90% and a Matthew's correlation coefficient of around 0.7 for non-ruminant species and higher than 0.95 for ruminant species. Other methods, such PLS-DA and Subspace Discriminant, also produced acceptable results and required less computational time. This study showed that spectral analysis of PAPs, based on diffuse reflectance spectroscopy, is a promising technique for differentiating between ruminant species and other terrestrial animal species. The technique may therefore offer accurate and fast analysis of large volumes of feed products, a necessary prerequisite for the lifting of the EU ban on non-ruminant processed animal proteins.

1. Introduction

Since the introduction of the total ban on the use of processed animal proteins (PAPs) to feed farm animals and the subsequent extension of the prohibition with the so-called anti-cannibalism ban [1], the European Commission has invested a great deal of time and effort in funding projects to provide a scientific evaluation of analytical methods that will help member states to enforce such bans. Thus, research undertaken over the last ten years, mainly within the framework of two EU-funded R&D projects (STRATFEED and SAFEED-PAP [2,3]), has scientifically demonstrated the advantages, disadvantages and complementarities of several methods, such as optical microscopy, NIRS, NIRS-microscopy, hyperspectral imaging (HSI), Polymerase chain reaction (PCR) and immunoassay techniques [4]. The outcome of this research has led to major advances in the area of standardising and validating the official method based on optical microscopy [5], as well as research into the potential of other methods that could complement or replace microscopy [6,7].

In 2010 the European Commission's TSE Roadmap 2 [8] started contemplating the possibility of lifting the ban on the feeding of processed animal protein derived from non-ruminants (e.g. pigs, poultry, fish) to non-ruminants of a different species and clearly stays that "it is of paramount importance to continue research in those areas where information is lacking or gaps exist which do not allow firm decisions to be taken". Among the methods that could provide a solution to the problem of differentiating between animal species, while simultaneously enabling the inspection of large volumes of PAPs, NIRS technology, either on its own or combined with microscopy (NIR-m) or HSI, undoubtedly occupies a place of major importance [9,7].

NIRS technology has already been installed in the animal feeds industry, both at the level of raw material suppliers and in the feed manufacturing industry itself. Leading European and world animal feed producers have developed global networks for NIRS analysis and many feed laboratories offer NIRS analytical services at approximately half the

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Table 1
Number of pixels (and samples) selected for each animal class for calibration (CAL) and validation (VAL).

		CAL	VAL	CAL + VAL
Class 1	Swine (SM)	9576 (38)	3780 (15)	13,356 (53)
Class 2	Poultry (PM)	9576 (38)	3528 (14)	13,104 (52)
Class 3	Ruminant (RM)	3780 (15)	1260 (5)	5040 (20)
Total		22,932 (91)	8568 (34)	31,500 (125)

cost of wet chemistry and a turnaround time of only 24 h [9,11]. As the technique is already widely in place in the feed industry and associated laboratories, it is the most likely to be used as the food chain's first line of defence. NIRS may provide an affordable first step in the control systems to be implemented by the industry and the inspection bodies in rendering plants and feed industries to ensure that the end-product complies with the prevailing regulations designed to mitigate against bovine spongiform encephalopathy (BSE) [9,12]. Despite all of the above-mentioned, only one study has been conducted to date to demonstrate the capacity of conventional NIRS technology to identify animal species in PAPs derived from rendering industries [13]. The authors concerned found that PLS discriminant models used to distinguish between ruminant and non-ruminant feeds classified 88.7% and 92.9% of ruminant and non-ruminant samples correctly, respectively, in the validation step. In a model consisting of three classes (poultry vs. pig vs. a mixture of ruminant PAPs), and also for the validation of the model, the classification rates were 96.2%, 92% and 86.3% respectively. However, the two class- and three class-models classified four out of 52 samples of ruminants as non-ruminant and nine out of 57 samples of the mixture of ruminants and other species as poultry. If NIRS is to be introduced on a large scale, it is imperative that the number of false negatives for ruminants be minimised.

Conventional NIRS technology can be viewed as single-point spectroscopy, where only the spectra of the whole target sample are of concern to spectroscopists. Thus the spatial distribution of different chemical compounds in the sample is lost and a minimal contribution to the bulk sample may go undetected by conventional NIR spectroscopy [14]. HSI cameras combine the advantages of spectroscopy and machine vision in addressing food quality and safety problems by providing full-spectrum data for every pixel in food-product images, enabling spectral and spatial analysis for correlation to composition, contaminants and physical attributes, such as size and shape [15]. Furthermore, compared to NIR-microscopy and conventional NIRS, the advantage of acquiring hyperspectral data at high spatial resolution is that the occurrence of mixed pixels is reduced, which may provide more consistent hyperspectral profiles and therefore increase the likelihood of accurate classification [16].

Fernandez-Pierna et al. [17] analysed animal feed particles in the 900–1700 nm range using HSI; however, in that study animal particles were differentiated from vegetable particles in animal feed and no discrimination was done between animal species. More recently, Nansen et al. [18] used hyperspectral profiles in 150 spectral bands (419–892 nm) covering a very short range of the near infrared region to detect bone meal in animal feeds. However, both these papers focused on the capacity of HSI and machine vision to detect terrestrial proteins in animal feeds. Riccioli et al. [2] also used HSI, in their case to distinguish terrestrial from fish meal in PAPs. They evaluated several algorithms for pixel selection in order to reduce the computational problems caused by the typically large databases produced by HSI instruments. The results showed that the various partial least squares-discriminant analysis (PLS-DA) models developed, with the pixels selected by each algorithm, achieved a successful classification rate in which more than 99% of samples were correctly identified as either terrestrial or fish meal.

Hitherto there has been no scientific evidence demonstrating the potential of HSI for detecting animal species in PAPs, something that would assist the European Commission in the process of taking decisions on lifting the ban prohibiting PAPs derived from non-ruminants being fed to non-ruminants of a different species. There is thus a clear need for greater scientific knowledge regarding any method that could ensure effective differentiation between animal species in the large volume of PAPs being produced and used in the huge number of rendering and feed plants operating throughout Europe and the world.

To meet the requirement of monitoring PAPs in real time, fast data acquisition and accurate and real-time image processing is needed for HSI to work appropriately [3]. However, a high spectral dimension also poses significant challenges to the analysis of hypercube. High dimensionality can significantly increase the computational burden and storage space. It is therefore desirable to make a careful selection of pixels for the calibration set and it is also extremely important to factor in the computational time required when selecting the discriminant algorithm. Most classification/discrimination research using NIRS and HSI data has employed the PLS-DA algorithm. This may be attributable in part to the widespread availability of this algorithm in the software packages that accompany the instruments. There are however many other straightforward statistical methods that can very easily be used either to augment PLS or as alternative supervised learning methods to PLS-DA [19].

For the purposes of this paper a set of rendered feed samples belonging to different categories (poultry, swine and ruminants) were used to compare the predictive capabilities of Random Forest (RF), Support Vector Machine (SVM), Partial Least Squares-Discriminant Analysis (PLS-DA) and Subspace discriminant (SSD) algorithms. All classifications are based on the PAPs' near infrared (NIR) spectroscopy data.

2. Material and methods

2.1. Samples

A total of 125 rendered meal samples collected over a period of three years and belonging to different categories and were analysed: 53 pure swine meals (SM), 52 pure poultry meals (PM) and 20 pure ruminant meals (RM). Further information about the samples is available in de la Haba et al. [13]. From the overall set of 125, 34 samples (15 from SM, 14 from PM and five from RM) were randomly selected and transferred from the calibration set to a validation set.

2.2. Hyperspectral image capturing and pre-processing

One gramme of each sample was used for the analysis. Samples were analysed with a NIR camera (MatrixNIR, Malvern Instruments, Maryland, USA) featuring an Indium Gallium Arsenide (InGaAs) focal-plane array detector capable of recording images of 240 × 320 pixels with a spatial resolution of 97.8 μm/pixel and a spectral resolution of 6 nm in the region 900–1750 nm. Only wavelengths between 1000 and 1700 nm were taken into account, thereby avoiding spectrum-tail noise. At around 900 nm, in fact, the detector is less sensitive and lower intensities are obtained because longer integration times are needed [2]. Images were cut to 216 × 280 pixels in order to eliminate anomalies near sample edges due to the fact that in many images the entire field of view was not covered by the sample.

Based on the results obtained elsewhere [2] using the same instrument and methodology, it was decided to employ the spatial interpolation method to reduce the size of the data set. The spatial interpolation function present in ISYS 4.0 software (Malvern, Inc., Olney, MD, USA) enables changing the density of points in an image cube across the two spatial dimensions using a bilinear interpolation function. In the present study one pixel was selected from among a 16 × 16 neighbourhood of known pixel values. Thus, a total of 252 pixels per each original image of 216 × 280 pixels [60,480 divided by 16 and by (16–1)] was obtained. This method ensured representation of the whole spatial variability of the sample due to e.g. possible differences in lighting or variations in sample thickness across the camera field of view. Table 1 gives details of the