Chemometrics and Intelligent Laboratory Systems 172 (2018) 90–99

Contents lists available at ScienceDirect

Chemometrics and Intelligent Laboratory Systems

journal homepage: www.elsevier.com/locate/chemometrics

Using spectral and textural data extracted from hyperspectral near infrared spectroscopy imaging to discriminate between processed pork, poultry and fish proteins

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ARTICLE INFO

Keywords:
Protein animal by-products
Near infrared hyperspectral imaging
Pixel selection algorithms
Partial least squares discriminant analysis
Spectral and textural analysis
Classification trees

ABSTRACT

This paper proposes a method based on Near Infrared Hyperspectral Imaging for discriminating between pork, poultry and fish species in processed animal protein meals. First, an investigation was conducted into the possible importance of incorporating into the discrimination models anomalous (or singular) pixels as probable discriminant pixels for each species. Subsequently, partial least squares discriminant analysis (PLS-DA) spectral and textural models were constructed. The former reflected the spectral information (spectral trace), and the latter the spatial (textural trace) information based on different groups of features. Finally, the spectral and textural information was integrated using classification trees, to ascertain whether the combined use of such information represented an improvement in accuracy in the effort to discriminate between species.

The method was applied to a set of 40 pork, 40 poultry and 40 fish meals analysed in the 1000–1700 nm range. Models were then tested using an external validation set comprising 45 samples (15 pork, 15 poultry and 15 fish meals). The results demonstrated that combining spectral and appearance characteristics in a single classification tree generated better classification results for the samples used in the study (92% correct) than when using the PLSDA spectral model (83% correct).

1. Introduction

Processed Animal Protein (PAP) is an EU term applied to rendered materials belonging to category 3 by-products (fit for human consumption). These can be derived from animal by-products (ABP) or land animal protein (LAP) and from fish. PAPs and LAPs are now highly valued by a number of industries, and have become a major complete-feed ingredient for pets [1].

Article 11 of Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009, prohibits the feeding of terrestrial animals of a given species (e.g. pork, poultry), other than fur animals, with PAP derived from the bodies or parts of bodies of animals of the same species (intra-species recycling). Article 11 also prohibits the feeding of farmed fish with PAP derived from the bodies or parts of bodies of farmed fish of the same species. As stated in its Strategy paper on Transmissible Spongiform Encephalopathies for 2010–2015 [2], the Commission recognizes that a lifting of the ban on the use of PAP from non-ruminants to non-ruminants could be envisaged, provided that the existing prohibition on intra-species recycling is maintained and only if validated analytical techniques to determine the species origin of PAP are available. Following similar lines, in 2013 the Commission adopted a first review of the feed ban provisions in order to allow aquaculture animals to be fed with PAP derived from non-ruminant farmed animals.

However, despite the huge amount of effort invested, there is still no validated diagnostic method capable of detecting the presence of porcine or poultry material in feed. Therefore, it would not be possible to control the correct implementation of the prohibition on intra-species recycling should the use of PAP of porcine origin in poultry feed or the use of poultry PAPs in pig feed be re-authorised [3]. In response to the regulatory pressure, the industries concerned, via their national and international associations, have made great efforts to collaborate in and finance scientific research [4]. With regard to the lifting of the feed ban on non-ruminant PAP-use within the EU – poultry PAP to pigs and porcine PAP to poultry – the European Feed Processors Rendering Association (EFPRA) is still waiting for Polymerase Chain Reaction tests that must be available before the EC will consider lifting the ban. The problem is that the tests cannot distinguish between legal ruminant

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https://doi.org/10.1016/j.chemolab.2017.11.011
Received 8 September 2017; Received in revised form 13 November 2017; Accepted 15 November 2017
Available online 22 November 2017
0169-7439/

ingredients, such as milk, and ruminant PAP. EFPRA has consequently claimed that this can easily be resolved by testing the ingredients – the PAP – rather than the finished feed, where more ingredients are mixed into the final diet.

One of the techniques that has shown the most promising results in distinguishing between animal species in animal-derived feeds is NIR microscopy combined with chemometric techniques. De la Haba et al. [5] were able to distinguish between particles of terrestrial animals by modelling their NIR microscopy data with a Support Vector Machine (SVM). Pérez-Marín et al. [6] were able to distinguish between particles of terrestrial and aquatic animals. In their case, the methodology proposed was based on the prior selection of animal particles and the subsequent application of a K-nearest-neighbours (KNN) model. This methodology showed its potential for detecting the presence of animal content in composite feeds. Other studies, such as that of Boix [7], went further in this direction. The greatest drawbacks implicit in this technique in terms of its industrial application are that it is highly time-consuming, requires specific training and involves the use of carcinogenic substances to extract the fragments under analysis [6]. Conventional NIR technology, overcoming these problems, has been used to determine the percentage of animal content in composite feeds, as well as to distinguish between feeds originating from different species. De la Haba et al. [8] distinguished, with a 90% success rate, not only between ruminant- and non-ruminant-based feeds, but also between poultry-, porcine- and ruminant-based feeds, although it should be pointed out that the error rates are high in terms of the detection limits stipulated by the EU.

One of the preliminary stages in any protocol involving chemometric treatment is discarding anomalous spectra [9]. The application of this to hyperspectral images at the level of pixels, whether or not representative pixels are chosen [10], incurs the risk of eliminating pixels that, despite their low proportional incidence, are exclusive to a particular type of feed and are therefore discriminant. Riccioli et al. [11] proposed a method based on hyperspectral images to distinguish between feeds derived from terrestrial animals and fish using a selection of representative pixels, achieving a rate of precision at the pixel level in excess of 99%. Riccioli et al. [10] compared the performance of classic NIRS spectroscopy with hyperspectral NIRS imagery in the detecting and quantification of ruminant feed in PAPs. The results showed that whereas the hyperspectral image had greater potential in terms of species differentiation, due to the qualitative information it provided, the quantification of contamination with ruminant feed was established with greater accuracy by classic NIRS.

Another method that has been investigated recently focuses on extracting distinctive geometrical features by analysing microscopic imagery. Pinotti et al. [12], tried to distinguish between poultry and mammal particles in animal feeds by extracting geometrical attributes. Although significant differences were detected between classes, it was only possible to make a distinction between averages, not individual particles, owing to the major overlaps between classes. Using the same method, Ottoboni et al. [13] found average differences between porcine and bovine particles, but the method proved unsuitable for differentiating between individual particles. Yao et al. [14] were able to distinguish between particles of fish and other animal meals on the basis of their geometrical properties. Discrimination between poultry and mammals proved to more difficult however, with a discrimination rate of 93% being achieved. Differentiation between porcine and bovine particles was not possible.

Studies into the more traditional application of multispectral image analysis in foods have focused on spectral signature rather than imaging features. Analysis of the hyperspectral image however enables a simultaneous focus on both spectral and geometrical aspects. Although applications of this geometrical approach have been limited to certain fields, such as the segmentation of aerial or satellite imagery to identify land use, they have also in recent years been identified as a valuable tool in agri-food areas [15].

A general procedure for extracting textural features of images in the spatial domain was presented by Haralick et al. [16]. A co-occurrence matrix (GLCM) is a square matrix with elements corresponding to the relative frequency of occurrence of pairs of grey level of pixels separated by a certain distance in a given direction (0°, 45°, 90° or 135°). The standard procedure extracts the textural attributes of monochrome images. When it is applied to a hyperspectral image, the extraction is usually also carried out using monochrome images: in a specific band or in projections on the principal components. In the realm of food technology, Garrido-Novell et al. [15] applied textural analysis to images of slices of ham projected onto the first principal component for qualitative analysis of the slices with a hyperspectral image. In the realm of remote sensing, studies such as that of Huang et al. [17] propose methods of applying textural analysis to multispectral and hyperspectral images, using alternative methodologies to the analysis of principal components.

This study is aimed at evaluating a methodology based on a model that combines spectral and texture information extracted from hyperspectral NIR imaging to improve accuracy in discrimination between processed pork, poultry and fish proteins.

2. Experimental

2.1. Image acquisition

A total of 120 meal samples belonging to different categories were analysed: 40 pure poultry meal samples, 40 pure swine meal samples and 40 pure fish meal samples. The samples contained particles of scale, hair, feather, blood, grease, skin, muscle, and bone from either fish or terrestrial animals and were obtained from several rendering plants.

One gram (1 g) of each sample was used for analysis. Samples were analysed with a line scan NIR imaging system comprising a camera (XEVA-1.7-320 CCD, Xenics, Leuven, Belgium), a spectrograph (ImSpector V10E, Specim, Oulu, Finland) ranging from 900 to 1700 nm, two 250 W halogen lamps and a control step platform (Velmex, Bloomfield, NY). The image size was 256 × 200 pixels with a spatial resolution of 0.5 mm^2/pixel and a spectral resolution of 3.3 nm.

A 2601-pixel Region of Interest (ROI) was selected in order to avoid those parts of the image not belonging to the sample (Fig. 1).

Raw images were transformed into reflectance images using a 99% reflectance ceramic board. A reflectance reference was obtained by

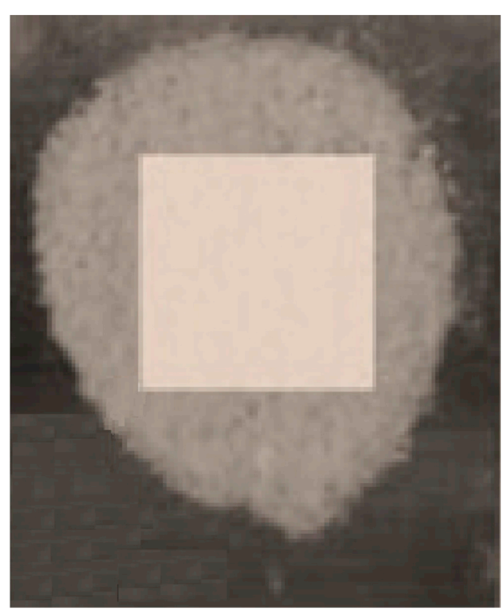

Fig. 1. Sample of meal on a steel plate and ROI.