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# Differentiation of Beef and Fish Meals in Animal Feeds Using Chemometric Analytic Models

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## Abstract

**Purpose:** The research presented in this paper applied the chemometric analysis to the near-infrared spectral data from line-scanned hyperspectral images of beef and fish meals in animal feeds. The chemometric statistical models were developed to distinguish beef meals from fish ones. **Methods:** The meal samples of 40 fish meals and 15 beef meals were line-scanned to obtain hyperspectral images. The spectral data were retrieved from each of 3600 pixels in the Region of Interest (ROI) of every sample image. The wavebands spanning 969 nm to 1551 nm (across 176 spectral bands) were selected for chemometric analysis. The partial least squares regression (PLSR) and the principal component analysis (PCA) methods of the chemometric analysis were applied to the model development. The purpose of the models was to correctly classify as many beef pixels as possible while misclassified fish pixels in an acceptable amount. **Results:** The results showed that the success classification rates were 97.9% for beef samples and 99.4% for fish samples by the PLSR model, and 85.1% for beef samples and 88.2% for fish samples by the PCA model. **Conclusion:** The chemometric analysis-based PLSR and PCA models for the hyperspectral image analysis could differentiate beef meals from fish ones in animal feeds.

**Keywords:** Beef, Chemometrics, Fish, Hyperspectral image, Line-scan, NIR

## Introduction

European Union (EU) regulated zero tolerance for the existence of meat and bone matters (MBM), as part of animal protein sources, in compound animal feeds to respond to the increasing demands to protect and ensure food safety and public health (Riccioli et al., 2011). The existence of banned matters in animal feeds could cause serious diseases, such as the bovine spongiform encephalopathy (BSE). To satisfy the demands from the regulations and the public health concerns, it is essential to automatically identify and differentiate the contents of animal feeds in the animal-feeds processing plants (Brookes, 2001; Riccioli et al., 2011). The non-destructive technology using machine vision for such automatic detection can be potentially the optimal promising solution to satisfy the demands of food safety and public health (He et al., 2014; June et al., 2013; Lee et al., 2014; Yang et al., 2014).

Among various non-destructive technologies of optical engineering, the hyperspectral imaging has been applied for food safety and quality purposes (Elmasry et al., 2012; He et al., 2014; June et al., 2013; Lee et al., 2014; Yang et al., 2010; 2014). Specifically, the hyperspectral imaging technology has been used in the attribute analysis for many animal food products, such as fish (He et al., 2014), beef (Elmasry et al., 2012), and poultry (Yang et al., 2010). To apply the hyperspectral imaging technology to the

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automatic detection in the food product processing line, line-scanning machine vision system has been developed and shown great potential for quick and reliable food safety inspection (Yang et al., 2010; 2014).

On the other hand, hyperspectral imaging in the line-scanning machine vision system could continuously and quickly produce huge quantity of spectral and spatial data for image analysis and classification. To quickly and properly analyze such a huge quantity of hyperspectral image data, chemometric analysis could be a reliable statistical tool to generating correlation models (Garrido-Novell et al., 2015). Therefore, the hyperspectral line-scanning machine vision system implemented with the chemometric-analysis-based model will be very useful and essential for the online application of food safety and quality inspection.

The fish meal is a high protein source of the compound animal feeds. It is important to detect banned animal matters, such as beef, in fish meals. Therefore, the primary object of this research was to apply the chemometric analysis to the development of the classification models using hyperspectral data to distinguish beef and fish meals in animal feeds. Each pixel of the Region of Interest (ROI) of the meal image was treated as an independent object for model development and evaluation. The main goal of the first step of the research reported in this paper was to correctly classify as many pixels of beef meals as possible for public health concern.

## Materials and Methods

This research collected 15 samples of beef meals and 40 samples of fish meals prepared in a rendering plant. The meal samples could randomly contain various materials, such as muscle, skin, blood, bone, grease, and feather. One gram of each rendered meal sample was retrieved and carefully spread in a black paper for line-scanning. The black paper was used as a dark background. The hyperspectral line-scan machine vision system was composed of a XEVA-1.7-320 CCD camera (Xenics, Leuven, Belgium), an ImSpector V10E spectrograph (Specim, Oulu, Finland), two 250-W halogen lights and a VXM stepper motor controlled platform (Velmex, Bloomfield, NY) (Garrido-Novell et al., 2015). The original size of an image was 320 × 256 pixels. The image resolution was approximately 0.5 $mm^2$/pixel. The camera and the spectrograph were built specifically for the near infrared spectral range, in which the hyperspectral data would present more useful information for proper classification of beef and fish meals.

While scanning the samples, they were placed on the platform and moved through the field of view of the camera to obtain line-scan images. Each image represented a scanned line along the field of view of the camera. Through the slit of the spectrograph, incoming light was dispensed into a spectrum for each pixel of the line image. Therefore, a line-scanned image contained spectral data along one and spatial data along another axis.

In this research, an original line-scanned image contained spectral data along one axis of 320 pixels, with a 3.3-nm spectral interval, and spatial data along another axis of 256 pixels. The trial-and-error-based preliminary study showed the need to discard 144 wavebands where the raw account of light reflectance from the scanned objects was too low or too noisy. Therefore, only 176 spectral-dimension pixels spanning from 969 nm to 1551 nm were used for data analysis and model development. Five meal samples were put on the platform and moved through the field of view of the camera to be scanned for 200 times.

The trial-and-error-based preliminary study also showed the need to identify a ROI in order to discard non-meal, dark background or improperly-imaged-meal. The size of ROI was manually selected to cover 3600 pixels for each sample. The images of eight beef meals and 20 fish meals, the development group, were used for hyperspectral data analysis and chemometric model development. The development group contained 28800 pixels for beef meals and 72000 pixels for fish meals. Each pixel would be an independent hyperspectral data source and differentiation object. The developed models were then applied to the images of the rest seven beef meals and 20 fish meals, the test group. The test group contained 25200 pixels for beef meals and 72000 pixels for fish meals. The models were evaluated using the results from the test group for the optimal model attributes.

After images were acquired, the line-scan images were converted from raw account to relative reflectance by the flat-field calibration with a reference white line image, *W*, and a reference dark current line image, *D*. Before meal samples were scanned, a white target with 99% diffuse reflectance, moving on the motor platform through the field of view of the camera, was scanned for four times. The acquired white line images were averaged to form the reference white line image. Afterward, the lens of the camera was covered in order to block all illumination from the environment to take another four shots. The acquired dark line images were averaged to form the reference dark current line image. With the averaged reference images $W$ and $D$, the raw hyperspectral images ($I_0$) of meal samples were calibrated and converted to the relative reflectance images ($I$) by the equation 1:

$$I = \frac{I_0 - D}{W - D} \tag{1}$$

The examples of calibrated images of beef and fish meal samples were shown in Figure 1. The intensity of the ROI in each image was increased intentionally for locating and viewing only. Within the ROI of five beef and five fish meal sample images randomly selected from the development group, the averaged spectra and the ranges of one standard deviation were calculated and shown in Figure 2. The hyperspectral graph in Figure 2 showed two highest spectral peak tops. The one in the shorter waveband was in the waveband of 1132 nm for beef and the top was slightly shifted to 1126 nm for fish. The one in the longer waveband was in the waveband of 1302 nm. In Figure 2, the reflectance for beef meals was generally higher than one for fish in wavebands shorter than 1302 nm. Specifically, the spectral difference between beef and fish meals could be observed easily in the spectral peak from 969 nm to 1192 nm. The spectral difference between beef and fish in 1132 nm, one of the spectral peak top, was much higher than that in 1302 nm, another spectral peak top. Such difference could be observed in Figure 1. On the other hand, the spectral difference was relatively low for the spectral range from 1192 nm to 1551 nm.

Although the spectral difference in Figure 2 was significant, such difference could not be used for the proper classification of beef and fish meals because the spectral variance was so high to possibly cause low success differentiation rate. High spectral variance was expected because the spectral reflectance from the meal samples could come from muscle, skin, blood, bone, grease, feather, or other materials of the rendered animal. To overcome this challenge, the chemometric analysis, a computationally fast statistical method ideally for complicated data modeling,

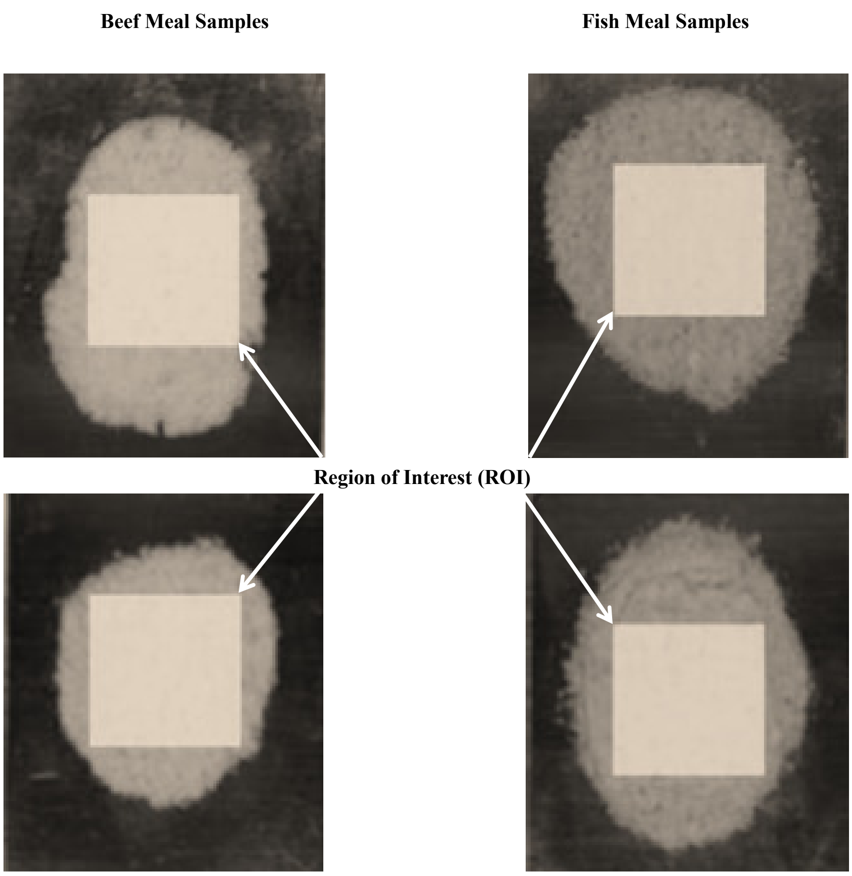


**Figure 1.** The samples for the line-scanned images of beef meals and fish meals with the Region of Interest (ROI) highlighted.


**Figure 2.** The spectra, in relative reflectance, for the hyperspectral images of beef meals and fish meals: the average spectrum plus one standard deviation (beef+std, fish+std), the average spectrum (beef, fish), and the average minus one standard deviation (beef-std, fish-std).

was applied to the development of the differentiation model.

Two most popularly used statistical tools of the chemometric analysis, the partial least squares regression (PLSR) and the principal component analysis (PCA), were applied to the model development. Both tools were reliable and robust for multivariate data analysis and modeling by generating components as new input variables to linearly compose original input variables (the relative reflectance in each waveband for this experiment). The components required for accurate model performance was usually much fewer than the original input variables. While generating the set of components to reduce dimension of the input variables, the PLSR intended to consider the impacts of outputs but the PCA would search the variance among the input variables. The details for the calculation and comparison of the PLSR and PCA can be referred to Godoy et al. (2014), Westerhuis et al. (1998) and Worley et al. (2013).

The models based on each tool were generated with components varied from one to ten for the optimal algorithm. The model required the relative reflectance from 176 wavebands from a pixel of the hyperspectral image for the inputs. The binary model output would determine the matter in this pixel to be beef or fish meal: zero for fish and one for beef. The total 100800 pixels of the development group were used for model development, and the total 97200 pixels of the test group were used for model evaluation. The models were developed, executed, and evaluated on the platform of MATLAB R2013a

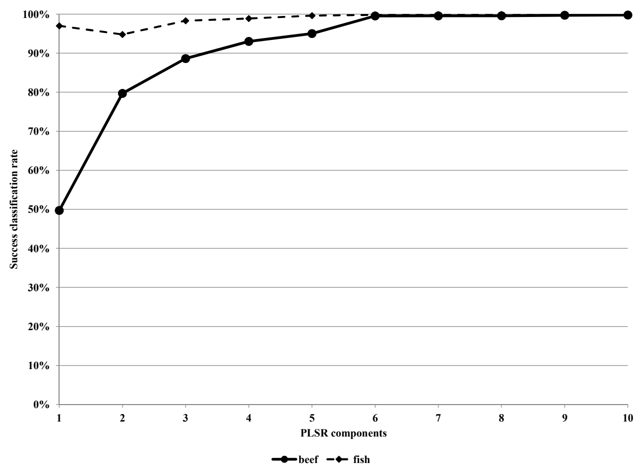


**Figure 3.** The results of the success classification rates for the PLSR models on the development group of 28800 pixels for eight beef meals and 72000 pixels for 20 fish meals.

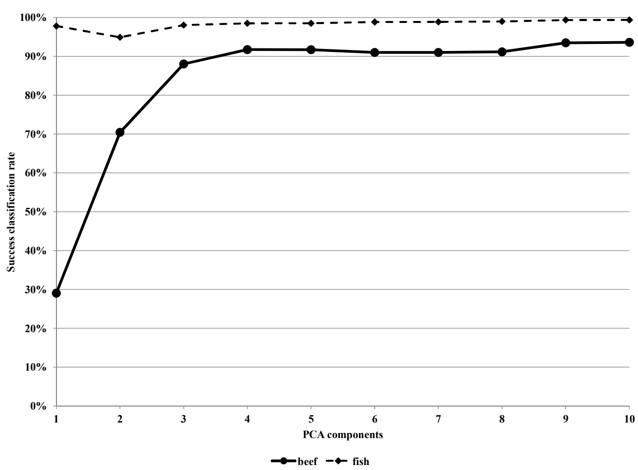


**Figure 4.** The results of the success classification rates for the PCA models on the development group of 28800 pixels for eight beef meals and 72000 pixels for 20 fish meals.

(MathWorks Inc., Natick, MA, USA).

## Results and Discussion

The results in Figure 3 showed that the PLSR models were generally developed well for the classification of beef and fish meals. With at least six components, the PLSR models could correctly classify more than 99% of pixels for both beef meals (28800 pixels in total) and fish meals (72000 pixels in total). The PLSR models misclassified less than 140 pixels for both beef and fish meals with at least six components, and even missed less than 100 pixels with at least nine components. The model performance was generally improved with more components during the model development.

The results in Figure 4 showed the similar well development for the PCA models. With at least four components, the PCA models could correctly classify more than 90% of pixels for beef meals and more than 98% of pixels for fish meals. Comparing Figure 4 with Figure 3, the PCA models could not perform as well as the PLSR models did during the model development. Regardless to the number of the components, the PCA models misclassified more than 1800 pixels for beef meals and more than 450 pixels for fish meals. However, less components could require less computational effort for the model to classify the pixels, which would be essential for online application for high-speed processing line. Because of increasing concerns for food safety, the possible economic loss caused by misclassification of fish meals in Figures 3 and 4 might be tolerable. It is more important to accurately detect beef matters due to zero tolerance for beef matters in fish meals; thus, the PLSR models performed much better than the PCA models during the model development. Nevertheless, both PLSR and PCA models showed significant potential for further analysis to differentiate beef matters from fish meals.

Figure 5 showed the PLSR models performed on the test group of beef meals (25200 pixels in total) and fish meals (72000 pixels in total). Similar to Figure 3, the PLSR models performed well with at least six components when the success classification rate was also higher than 99% of pixels for fish meals, and could correctly classify more than 96% of pixels for beef meals. The highest success classification rates were obtained by the PLSR model with nine components, 97.9% of pixels for beef meals (527 pixels misclassified) and 99.4% of pixels for fish meals (420 pixels misclassified). The high success classification rate for the pixels independent from the ones used for model development indicated that the PLSR models could be used in the real online application for food safety. The PLSR model would be built with six components when the computation speed is more essential, or with nine components when the most accurate identification of beef matters is required to exchange the computation speed.

Different from Figure 5, the results in Figure 6 showed that the PCA models could not successfully classify more than 90% of pixels for both beef and fish meals with the same model algorithm. With at least four components, the PCA models would successfully classify more than 81% of pixels for beef meals and more than 86% of pixels for fish meals. Although the results in Figure 6 was not as satisfactory as ones in Figure 5, the high success classification rates in Figure 4 implied that the performance of the PCA models could be improved with more data presented to the development group. This possible improvement could also be applied to the PLSR models.

Moreover, the samples were not scanned on the same day. Fifteen samples of beef meals were scanned on three days and 40 samples of fish meals were scanned on eight days, respectively. Considering the possible variance in the imaging environment and labor effort, the high success classification rates in Figures 3 to 6 showed that both PLSR and PCA models could perform well for time-series data. It is essential since the models were expected to be applied to the real online application for the future

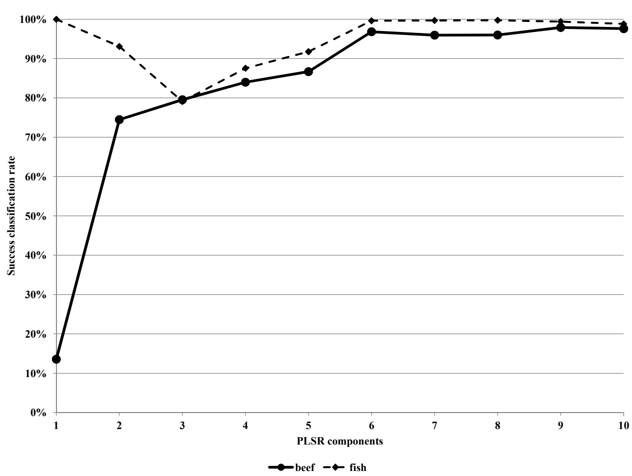


**Figure 5.** The results of the success classification rates for the PLSR models on the test group of 25200 pixels for seven beef meals and 72000 pixels for 20 fish meals.

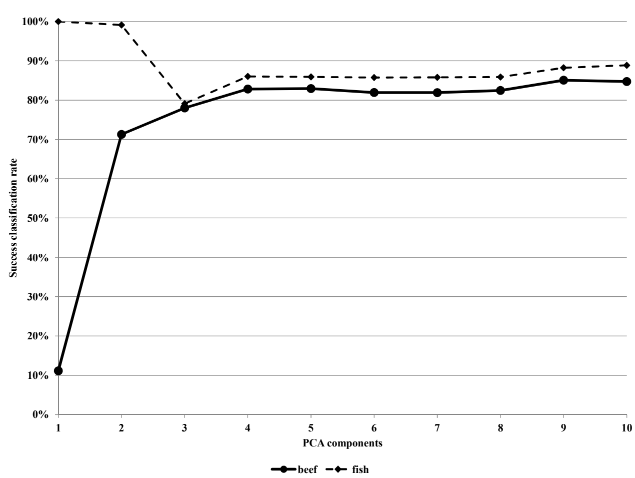


**Figure 6.** The results of the success classification rates for the PCA models on the test group of 25200 pixels for seven beef meals and 72000 pixels for 20 fish meals.

development.

The results showed the great potential applying the chemometric analysis-based algorithms, such as the PLSR and the PCA models, to the hyperspectral image analysis in order to differentiate beef meals from fish ones in animal feeds. The well-developed chemometric models could potentially handle high spectral variance caused by different materials of the rendered animal in the same meal. To further improve the model performance, more images would be collected for model development. Also, more proper illumination scheme would be designed for the line-scan machine vision system.

## Conclusion

This research reported the development and the evaluation of the chemometric analysis-based models using the partial least squares regression (PLSR) and the principal component analysis (PCA) methods for the differentiation of beef and fish meals in the line-scanned hyperspectral images. The hyperspecral data from 176 wavebands spanning from 969 nm to 1551 nm were used for model inputs. With nine components in the model algorithm, the success classification rates were 97.9% for beef meals and 99.4% for fish meals by the PLSR-based model, and 85.1% for beef meals and 88.2% for fish meals by the PCA-based model. High success classification rates indicated the greatly potential application of the hyperspectral line-scan machine vision system implemented with the chemometric analysis model for the detection of beef matters in fish meals. The system would provide essential help to ensure food safety and public health.

## Conflict of Interest

The authors have no conflicting financial or other interests.

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