Detection of pork freshness using NIR hyperspectral imaging

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ABSTRACT

In this study the potential of a pushbroom hyperspectral imaging system in the near-infrared (NIR) range (900-1700 nm) as a non-destructive technique was investigated for the detection of freshness in pork meat. Pork samples from the \textit{longissimus dorsi} muscle of different quality grades were investigated. Each sample was imaged by the hyperspectral imaging system at 2 days post-mortem and then kept frozen for six months. Samples were then removed from the freezer, thawed and imaged again by the system. The acquired hyperspectral images for both fresh and thawed samples were calibrated, the main region of interest (ROI) was identified and the spectral data were extracted. The spectral data were analyzed by multivariate analysis and the most important wavelengths were identified by \textsuperscript{2}nd derivative of the raw spectra. Results revealed that hyperspectral imaging technique has an excellent ability as a fast and non-destructive method for fresh pork authentication.

Keywords: Hyperspectral imaging; Meat; Frozen pork; Principal Component Analysis; Near-Infrared (NIR)

INTRODUCTION

Meat is a highly perishable food commodity, and requires special attention to keep its quality and safety for consumption. Low temperatures slow down quality deterioration caused by growth of spoilage bacteria as well as chemical and biochemical changes. Chilling also keeps harmful bacteria from growing and chilled pork requires a high standard of disease control and sanitation. Freezing is recognized as a safe way to preserve meat for longer periods. However, meat quality declines during frozen storage, and frozen meat does not have the same quality as fresh meat. Most meat shows a gradual deterioration in quality attributes with frozen storage such as increased drip losses and decreased protein extractability. Hence, it becomes dry and less tasty because juices and micro nutrients are carried away together with the ice water during thawing. The risk of damage caused by the formation of ice crystals affects not only the structure of cell membranes but also causes protein denaturation, which is favoured by the increase in ionic strength due to fibre dehydration. It has been suggested that the fluctuations in temperature that frequently occur during storage and transportation of frozen foods cause re-crystallisation phenomena and could explain the observed deterioration during frozen storage [1].

The quality decline is exacerbated by the rate and conditions at which meat is thawed. This increased drip loss also causes the loss of thiamine and folates amongst others, and significantly downgrades appearance of meat. After thawing, meat often does not oxygenate back to the same level as before freezing. This lack of oxygen darkens the meat and this darkening gets worse the longer the meat is frozen reflecting poorer quality [2]. In Europe, the main authenticity issues dealing with fresh meat are origin, breed within a species, irradiation, previous freezing and ageing [3]. Fresh meat has a higher market price than thawed frozen meat, and many retailers sell thawed frozen pork as chilled fresh meat. Since both fresh and frozen-thawed meat look the same, identifying fraud retailers is a troublesome task.

In order to prevent meat retailers from offering thawed, imported frozen meat as fresh domestic ones, various methods have been proposed in the past for identification of frozen and thawed meat [4]. Most common methods rely on electrical properties [5-6] and enzyme activity determination [7-9]. Recently, it is proved that the suitability of spectroscopic methods for identification of frozen and thawed beef in the NIR and visible range [10-12]. In some studies about correct classification of 95\% was obtained by collecting spectra in the range 650-1 100 nm from intact beef slices [11], while even better results (100\% correct classification) by scanning between 1100 and 2500 nm were achieved [10]. The thawing behaviour of frozen chicken without exposure to air was investigated by Vis/NIR spectroscopy. Results suggested that the melting of ice crystals and the relaxation and proteolysis of proteins precede the relaxation of lipids, since the O–H/N–H
bands change their spectral intensity before the C–H groups [13]. It is advantageous to use near-infrared to provide more detailed spectral information, since hyperspectral systems operating in the visible region (400-800 nm) have considerably weaker band intensities when compared to the NIR range (900-1800 nm). Hyperspectral imaging is a rapid, non-destructive and reagent-less analytical technique that integrates both spectroscopic and imaging techniques in one system for providing both spectral and spatial information simultaneously. It provides spatial information, as regular imaging systems, along with spectral information for each point in the image as spectroscopic techniques.

In this study, the overall objective was to investigate the ability of NIR hyperspectral imaging technique for accurate and objective classification of pork samples according to its freshness. Specific objectives were to:

- Extract spectral information and identify optimal wavelengths most related to pork freshness;
- Apply multivariate analysis to identify and differentiate fresh from frozen-thawed pork samples;
- Develop an image processing algorithm to transform the spectral images into pseudo-colour images using the results obtained from the multivariate analysis.

**MATERIALS & METHODS**

**Sample preparation**

A set of 15 fresh pork chops (one inch in thickness) from the *longissimus dorsi* muscle was used in this study. Fresh chilled samples (2 days post-mortem) were imaged in the hyperspectral system. After imaging, the samples were vacuum-packed and frozen in a commercial freezer. After 6 months, the samples were thawed for two hours at room temperature and imaged again.

**Hyperspectral image collection**

Figure 1 shows the main configuration of the pushbroom hyperspectral imaging system. The system consists of spectrograph (ImSpector, N17E, Spectral Imaging Ltd, Finland), a camera (Xeva 992, Xenics Infrared Solutions, Belgium), illumination source (tungsten-halogen V-light, Lowell Light Inc, USA), a conveyer (MSA15R-N, AMT-Linearways, SuperSlides & Bushes Corp., India) and a computer with data acquisition software (SpectralCube, Spectral Imaging Ltd., Finland). The spectrograph had a fixed-size internal slit (30 µm) to define a field of view (FOV) for the spatial line (horizontal pixel direction) and collected spectral images in a wavelength range of 900-1750 nm with a total of 256 bands. The conveyer was driven by a stepping motor (GPL-DZTSA-1000-X, Zolix Instrument Co, China) with a speed of 2.7 cm/s.

![Figure 1. Hyperspectral imaging system used for image acquisition](image)

During image acquisition, each pork sample was transported by the conveyer to the field of view (FOV) of the camera, where image was taken and stored in the computer. The image acquisition process was controlled by the SpectralCube software and one hyperspectral image was acquired for each sample.

**Image pre-processing**

The acquired image consisted of 237 bands ranging from 910 to 1700 nm. To correct the acquired images ($I_0$) from the dark current of camera, dark and white hyperspectral images were also acquired. Both images were used to calibrate the original hyperspectral images. A corrected reflectance image (I) was calculated with Equation:
where $W$ was the reference image obtained from a white reference tile and $B$ was the dark current image acquired with the light source off and a cap covering the lens.

A region of interest (ROI) was selected comprising only the loin eye area. The loin eye average spectral information was used for comparison between fresh and frozen-thawed samples. For each image, a mean reflectance spectrum of the ROI was calculated by averaging the spectral responses of all pixels in the ROI. In total, 30 mean reflectance spectra were obtained, one for each sample fresh and after thawing. ROI selection and reflectance spectra extraction from the hyperspectral images were performed using ENVI 4.6.1 (ITT Visual Information Solutions, Boulder, CO, USA).

**Data analysis**

Second derivative was performed for the extracted spectral data of the two conditions to identify the main wavelengths that could explain the difference among samples. PCA is a form of factor analysis that seeks a linear combination of variables such that the maximum variance can be extracted from data. It then removes this variance and seeks a second linear combination which explains the maximum proportion of the remaining variance, and this procedure is repeated several times, resulting in orthogonal (uncorrelated) factors. It is commonly used to reduce a large number of variables to a smaller number of factors for data modelling or to select a subset of variables from a larger set. In this study, principal component analyses were performed on the spectral data of pork samples and the resulting loadings were then used to extract the useful information attributed to difference in pork freshness. The data analysis was executed using multivariate analysis software (Unscrambler version 9.7, CAMO, Trondheim, Norway).

**RESULTS & DISCUSSION**

The typical reflectance spectra of the analyzed fresh and thawed samples are shown in Figure 2. Each spectrum represents the average for the loin eye area of the fresh and frozen-thawed samples. The different spectra showed similar patterns, but differ on the reflectance absolute values mainly in the range from 970 to 1000 nm and from 1150 to 1700 nm. Fresh samples showed the lowest reflectance values along the spectra, while thawed samples had higher values.

![Figure 2. Average reflectance spectra for fresh and frozen-thawed samples.](image)

Second derivative average spectra allow for the visualization of greater spectral detail. The derivatives show the location of maximum spectral variance, where more important features at 961, 1071, 1124 and 1147 are identified as shown in Figure 3. These wavelengths were used to build a PCA model to identify fresh and frozen-thawed pork meat with reduced data processing.
The first three principal components were responsible for 99.98% of variability of the data; the first, second and third principal components variability were 99.22%, 0.61% and 0.15%, respectively. As shown in figure 4, almost all thawed samples are located in the positive area of PC2, while the fresh samples are almost entirely located in the negative area. The principal components method is able to differentiate pork samples based on the reflectance values obtained from a reduced number of wavelengths.

The results of multivariate analysis model from the extracted spectral data can be transferred to each image to recognize whether it is fresh or has been previously frozen in a non-destructive manner. In this study, the first three PCs obtained from the spectral data of pork samples were used for generation of PC score images, as they accounted for more than 99.9% of the variance in the spectral data. The pseudo-colour image is obtained by multiplying the PC loadings by the spectral images in the selected wavelengths. Thus, pixels in the composed pseudo-colour image having same features tend to be indexed with the same values in the colour map. As a result of conversion to pseudo-colour image, fresh samples are presented in blue colour, while frozen-thawed samples are presented in red. Figure 5 shows the pseudo-colour images for the same samples before freezing and after thawing. Accordingly, the first row shows the fresh samples and the second row show the respective frozen-thawed samples. Detailed observation of these samples show that there are regions of different classes in certain parts of the loin eye, but these regions are small compared to the larger regions which determined the sample freshness. The method allows for the further classification of samples based either on their spectral information, as shown by the PC scores plot, or by the number of pixels from
the pseudo-colour images. The method could be further enhanced by including a larger amount of samples. All the image post-processing steps described were carried out in a program written in Matlab (The Mathworks Inc., Natick, MA, USA) by the authors.

**CONCLUSION**

The study demonstrated the potential of NIR hyperspectral imaging coupled with principal components analysis for evaluating freshness of pork. The fresh and frozen-thawed pork samples could be distinguished by the respective spectral attributes. PCA was used to extract useful image features allowing for the differentiation of fresh and thawed pork. Optimal wavelengths were identified using second derivative transformation. The selected wavelengths could be potentially utilized by a multispectral imaging system for identifying fresh pork meat in real time. Further work will be performed to build a more robust model by including a larger amount of samples, and improve image processing and classification based on the selected optimal wavelengths. Customers could benefit from a fast and non-invasive system to guarantee meat freshness.

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