Spectral and Hyperspectral Inspection of Beef Ageing State

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1. Introduction

Near-infrared (NIR) spectroscopy measures the absorption of electromagnetic radiation at wavelengths in the range 780-2500 nm. NIR spectra of foods comprise broad bands arising from overlapping absorptions corresponding mainly to overtones and combinations of vibrational modes involving C-H, O-H and N-H chemical bonds. The concentrations of constituents such as water, protein, fat and carbohydrate can in principle be determined using classical absorption spectroscopy. However, for most food samples, this chemical information is obscured by changes in the spectra caused by physical properties such as the particle size of powders. This means that NIR spectroscopy becomes a secondary method requiring calibration against a reference method for the constituent of interest. As a consequence of the physics of diffuse transmittance and reflectance and the complexity of the spectra, calibration is normally carried out using multivariate mathematics.

NIR spectroscopy is used routinely for the compositional, functional and sensory analysis of food ingredients, process intermediates and final products. The major advantage of NIR is that usually no sample preparation is necessary. The analysis is very simple and very fast and can be carried out on-line. One of the strengths of NIR technology is that it allows several constituents to be measured concurrently (Encyclopedia of Analytical Chemistry).

First successful food applications of NIR spectroscopy were the quick quantitative determination of macro components (water, fat, protein, starch, etc.) of food and agricultural products. Its success was promoted by fast development of the areas of hardware and software. As important evaluation methods can be mentioned the MLR (Multiple Linear Regression), PLS (Partial Least Squares regression) linear methods and ANN (Artificial Neural Network) non-linear method. Recently qualitative methods (classification, detection) have come into consideration.

In case of cereal and cereal products, NIR has been used for e.g. the quality testing of crossbred material from wheat breeding programs, monitoring flour quality or other raw ingredients. In biscuit and confectionery bakeries, other ingredients such as sucrose, chocolate etc. may also be analyzed. NIR has been also used for compositional analysis of intact biscuit and bread dough pieces to control e.g. bread dough mixing stage. (Osborne 1984; Wesley 1998)

NIR has a key role in the analysis and process control of dairy products along with analysis of protein, moisture, fat and lactose contents (liquid milk, dried whole milk, skim milk and whey powders, cream, traditional and processed cheese). In case of fruits and vegetables, Japanese researchers have demonstrated a successful application of NIR to the on-line determination of sugar content in intact peaches and mandarins and developed an automated fruit sorting machine based on this principle (Kawano et al. 1992). In case of carrots, qualitative characteristic were measured and investigated by NIR techniques. (Firtha et al. 2006; Kaszab et al. 2008)
The characteristics of raw meats are influenced by a variety of factors (Yongliang et al. 2002), e.g. animal (breed, sex, age), environmental (feeding, transporting and slaughtering condition), and processing (storing time, temperature condition). During the ageing, beef muscles undergo several changes that can affect their quality. These changes are reflected in many characteristics such as colour, tenderness, flavour and juiciness.

Existing techniques in meat quality assessment, either instrumental (for example, Warner-Bratzler shear force measurement and Hunter colour measurement) or sensory evaluation can provide reliable information about meat quality. However, these techniques are destructive, time consuming, and unsuitable for on-line application. The development of fast, non-destructive, accurate, and on-line/at-line techniques is desired. Near infrared (NIR) spectroscopy could form the basis for such techniques due to the speed, ease of use, and less interferences from moisture or colour of meat samples. Over the years, NIR spectroscopy has been developed and applied considerably in quality management of beef meat products.

Fat, water and protein contents in industrial scale meat batches were determined on-line by near infrared (NIR) reflectance spectroscopy. The quantitative analysis models of the above constituents in pork meat were developed by using partial least squares (PLS) regression with internal cross-validation and optimized through spectra pretreatment and spectra region selection. The correlation coefficients ($R^2$) of the calibration models of fat, protein and water contents based on integrating sphere diffuse reflectance were 0.902, 0.919 and 0.901, respectively (Tøgersen 1999). NIR is a promising technique for large-scale meat quality evaluation. Near infrared reflectance spectra enabled the classification of pork longissimus muscles correlating with water-holding capacity (Geesink 2003).

NIR is usually used for the assessment of major components (e.g. protein, fat, moisture) in meat. According to the general absorption spectra of beef, there are certain ranges that refer to the moisture, fat and protein content. (Figure 1.) These ranges are about at 1200 nm and 1400 nm in case of fat. The protein content is referred at approx. 1500 nm. (Rosenvold 2007) The significant wavelengths are 1190 and 1450 nm, where water shows the maximum absorption at 20 °C. (Curcio et al. 1951).
Prieto et al. (2009) concluded that NIR was much better at predicting meat composition (fat, protein and water) than meat quality (marbling grade, tenderness etc.). Other derived properties, like the chemical changes of aged beef might be also detected by spectral measurement. However, because of the non-homogeneous structure (marbling) of beef, this method needs destructed samples to measure.

Conventional imaging (image processing and analysis) is a novel technology for recognizing objects and extracting quantitative information from digital images in order to provide objective, rapid, even remote sensing, non-contact and non-destructive quality evaluation.

In recent years in particular, computer vision has attracted much research and development attention: as result, rapid scientific and technological advances have increasingly taken place regarding the quality inspection, classification, and evaluation of a wide range of food and agricultural products.

The hardware configuration of computer-based machine vision system is relatively standard. A typical vision system is consists of an illumination device, a solid-state CCD array camera, a frame-grabber, a PC or microprocessor system and a high-resolution colour monitor. In case of image processing, after pre-processing (image-correcting, removing noise, applying smooth filters/contrast enhancing/scaling and equalising image histograms), the images are segmented as separating images into various regions in which the pixels have similar image characteristic. A large number of segmentation techniques have been developed. Of these, thresholding-based, region-based, gradient-based, and classification-based segmentation are the four most popular techniques in the food industry, yet none of these can perform with both high accuracy and efficiency across the wide range of different food products. (Zheng - Sun 2008)

In computers, images are stored and processed in the form of matrices. Elements of the matrices are referred to as pixels, in which two types of information are presented – geometric information (i.e. the location of the pixels) and surface information (the intensity values associated with pixels). From the geometric information, two different object measurements can be obtained: size and shape. From the surface information, colour and texture can be extracted. (Zheng - Sun 2004)

Hyperspectral imaging (HSI) is a non-contact, cutting-edge analytical technology that combines conventional digital imaging and spectroscopy in a single system. 20 years ago HSI was first developed to remote for satellite sensing on the earth. Hyperspectral imaging provides three-dimensional information from the analyzed objective; it can serve us for spatial and spectral information by each pixel of the recorded image. HSI provides a big amount of information in form called “hypercube”. In case of proper analysis, it can be applied for reliable characterizing of a certain object.

Hyperspectral imaging offers a new way for studying different tissues, but this measurement is much more influenced by the surrounding conditions. Proper calibration method of the sensor and particularly well-defined conditions are needed to reduce the noise and stabilize the measurement.

It has a big potential with discrimination, classification and detecting defects applied in quality control for the food industry.
In case of *strawberry*, HSI was found to be able to detect bruises at likely selected wavelength of 980 nm because of the increased and localized absorption of light at that wavelength by the expelled water from bruised tissues. (Nagata et al. 2006)

Hyperspectral spectroscopic technique was also used the ripeness of *tomatoes* (Polder et al. 2003). Hyperspectral imaging technology was studied for defect inspection of *pickling cucumbers*. The technique showed good detection accuracies (>90%) for bruises on the pickling cucumbers, and defect detection was found to be efficient by HSI at 800 nm. (Ariana et al. 2010)

Hyper-spectroscopy technique for the measurement of moisture and fat contents of breaded-fried chicken nuggets was investigated by A. Yavari et al. in 2009. Partial least squares (PLS) calibration models were developed for quantitative evaluation of the two parameters. R² and root mean square error (RMSE) for each prediction were calculated to assess the prediction capability of the model. R values of 0.92 were obtained from cross validation calibration for total moisture and fat contents.

According to Naganathan et al. in 2007, a visible/infrared hyperspectral imaging system was developed to predict tenderness of 14-day aged, cooked beef. A push-broom hyperspectral imaging system (wavelength range: 400–1000nm) with a diffuse-flood lighting system was developed and calibrated. The aim was to predict three beef tenderness categories, namely tender, intermediate and tough. With a leave-one-out cross-validation procedure, the model predicted the three tenderness categories with a 96.4 % accuracy and all of the tough samples were correctly identified.

**Aged beef** is more tender and flavourful than beef that has not undergone the aging process. Generally, beef is aged commercially with tightly controlled and monitored temperatures and humidity levels. Aged beef has increased tenderness because the muscle and collagen in the meat begins to change due to the work of certain enzymes. Maximum tenderness can be reached at around 11 days after the cow is slaughtered. Beef can either be dry aged or wet aged. Ageing is usually preferred because moisture levels are reduced to give the piece of meat a “beefier” taste and increased tenderness. In the alternative, wet aged beef is aged in a vacuum packed and sealed bag. It is a quicker method of ageing and it keeps the moisture levels of the meat high. Since the weight of the beef is retained, it is more cost effective than dry ageing. (http://www.wisegeek.com/what-is-aged-beef.htm)

Lately aged, tender beef is getting popular on the Hungarian market, the import (e.g. Argentine and Irish) aged beef pieces are already available, but their high price (ageing process provides added values) limits their general use (Vén 2010).

**2. Objective of the study**

One of the greatest challenges in the meat industry is to obtain reliable information about meat quality during post-mortem along the production process, ultimately providing guaranteed quality of beef products for the consumers.

The objective of this study was to estimate the ageing time by NIR absorption spectra and reflected hyperspectral data in case of beef sirloin samples. In addition, we investigated the location of the certain slices if their original position can be predicted using spectral data. First the stability of both measurement method were checked, the effect of open air was
examined to the meat surface. In the second measurement series the two measurement techniques were compared.

In case of the **stability experiment** the reproducibility of meat measurement was checked by inspecting *fresh-cut* and *outer surface* of beef sirloin samples, which were stored in different conditions (*O₂* and *vacuum-packed*). The samples were inspected by MetriNIR spectrophotometer.

In case of the 4-week **ageing experiment** ageing state and location of beef sirloin slices were estimated by NIR spectral and hyperspectral properties. All of the slices were measured by conventional spectrophotometer and hyperspectral system as well to compare their capabilities.

### 3. Materials and Methods

The measurements could be divided into two parts. Multiple stability experiments and an ageing experiment were performed.

The reproducibility of meat measurement was checked first as the **stability experiment**. The materials were 8-8 independent beef sirloin samples by each stability measurements. Fresh-cut and outer surface stored for three days in different conditions were inspected

- on *fresh-sliced surface of vacuum-packed* beef sirloin samples
- on surface of *vacuum-packed* beef sirloin samples after opening the package (without cutting)
- on surface of *oxygen-packed* (packages filled with 100 % *O₂*) beef sirloin samples after opening the package (without cutting)

The samples were left on open air during the 7 times 10 minutes of the measurements, every sample was inspected per 10 minutes, all for 70 minutes, and absorption spectra were recorded.

For the **ageing experiment**, a **whole beef sirloin** (length approx. 60 cm) was sliced into 20 equal slices. These slices were aged vacuum-packed for 4 weeks at 5 °C in stabe air-conditioned storage room. Each week 4-4 slices were inspected from fore rib to the direction of the rump (no. 1, 6, 11, 16 at first, etc.), and NIR spectra and hyperspectral data were recorded (Figure 2.).
3.1. Sample Preparation

In case of the stability experiments, after opening the vacuum- or the O$_2$-packages (Figure 3.), a sample was taken from each slice for the sample case of the MetriNIR spectrophotometer. In case of inspecting fresh-cut surfaces, a fresh-sliced piece of the vacuum-packed slices was taken.

In case of the ageing experiment, fresh-cut surfaces were inspected alone. After opening the vacuum-packages, the aged beef slices were cut into half parts parallel with the outer surfaces, and then both of the half-slices were straight-cut into half again, so 4-4 fresh-cut surfaces could be measured by each item. (Figure 4.) The samples were measured by MetriNIR spectrophotometer and HeadWall Hyperspectral Imaging System.

There was no pre-conditioning; the surfaces were inspected immediately after taking out them of the 5 °C-temperature ageing process.
3.2. Measurements

The spectra of the samples were taken using MetriNIR spectrophotometer and HeadWall Hyperspectral Imaging System (Figure 5.). The spectrophotometer works in the range of 740-1700 nm with 2 nm spectral resolution. The hyperspectral system works in the range of 900-1700 nm with 5 nm spectral resolution. The Indium-Gallium-Arsenide (InGaAs) sensor matrix had 256 px in spectral and 320 px in spatial direction. The altitude of sensor and the lens resulted in 0.46 mm spatial resolution.

Software environment of push-broom hyperspectral system was developed for controlling sensors and Y-table stepping motors. It supports the setting of optimal AD parameters, two-point spectral calibration, moves the table and acquires frames as to avoid spatial distortion in the hypercube. The hypercube retrieved is saved in ENVI file format for later image processing (ENVI) and statistical analysis (R Project).

- The first panel of the controlling environment (Figure 6.) helps to set optimal AD parameters of the sensor. In case of proper parameters, the histogram of signal shows a wide distribution within the range.
• The second one helps spectral and spatial calibration, setting the spectral and spatial region of interest. The pure signal and the relative or absolute reflectance can be displayed or saved. After setting the Y length of object, a measurement can be started moving the table and grabbing the frames synchronously.

• On the third panel, a hypercube is displayed. A channel or a pseudo-image of three channels or the linear combination of channels can be shown. This last mode can be very useful to quickly enhance certain properties on surface.

Figure 6. Screenshot of Argus hyperspectral data acquisition software

4. Hyperspectral image processing by ENVI algorithm

The reflected hyperspectral data were preprocessed by ENVI (Environment for Visualizing Images) algorithm. The Spectral Angle Mapper (SAM) supervised classification method was used for segmentation of the areas of the meat and the fat tissues on hyperspectral frames. SAM is a physically-based spectral classification that uses an $n$-dimensional angle to match pixels to reference spectra. The algorithm determines the spectral similarity between two spectra by calculating the angle between the spectra, treating them as vectors in a space with dimensionality equal to the number of bands. SAM compares the angle between the endmember spectrum vector and each pixel vector in $n$-dimensional space. Smaller angles represent closer matches to the reference spectrum. Pixels further away than the specified maximum angle threshold in radians are not classified (ENVI User’s Guide).

5. Data analysis of both type of measurements by MS Excel and R Project

Recorded absorption spectra of the stability and the ageing experiments were imported, processed and visualized by graphs based on MS Excel sheets.
The spectral data were transported from XLS to TXT file format, and were processed by R Project using *PLS regression* technique. PLS regression is based on linear transition from a large number of original descriptors to a small number of orthogonal factors (latent variables) providing the optimal linear model in terms of predictivity. The prediction of ageing time and location (variable Y) from spectral data (the wavelengths are variables X) requires special data structure for PLS regression. (Figure 7.) Using *cross-validation method* and considering the minimum RMSEP (Rout Mean Square Error of Prediction) values, different factors were used for calibrating models. The PLS regression models can be characterized with their determinants.

Figure 7. Data structure required by the PLS regression

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Location [no. of slice]</th>
<th>Ageing time [week]</th>
<th>740 nm</th>
<th>...</th>
<th>...</th>
<th>xₙ</th>
<th>...</th>
<th>...</th>
<th>x₄₈₁</th>
<th>1700 nm</th>
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6. Results

6.1. Results of the instrumental measurements

In case of the instrumental measurements, spectra were imported, processed and visualized by MS Excel sheets. The intensity was calculated as the integral values of absorption spectra with the areas under certain curve.

According to the *stability experiment*, in case of *fresh cut surface* the absorption spectra usually represented the same decreasing, brightening characteristic by the 7 times 10-minute measurements. The two significant wavelengths (at 1190 and 1450 nm) referred to the moisture content, where the absorption got lower gradually. (Figure 8.) Inspecting the intensity, the integral values of absorption spectra represented the same slow-decreasing characteristic. (Figure 9.)
Figure 8. Absorption spectra of a fresh cut surface during the 7 times 10-minute measurement, stability experiment

![Absorption spectra of a fresh cut surface during the 7 times 10-minute measurement - stability experiment](image)

Figure 9. The integrals of absorption spectra on fresh-cut surface of vacuum-packed samples

![Integral values of absorption spectra on fresh-cut surface stability experiment](image)

The intensity of the absorption spectra on the outer surfaces were darkened at the first 10-20 minutes, then the spectra remained stable. (Figure 10. and 11.)
According to the results of PLS regression, even after 70 minutes, samples and time of leaving them on open air could be identified well by their spectra. (Table 1.)

<table>
<thead>
<tr>
<th></th>
<th>Fresh-cut surface</th>
<th>O$_2$-packed surface</th>
<th>Vacuum-packed surface</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>prediction</td>
<td>$R^2$</td>
<td>RMESP</td>
</tr>
<tr>
<td>time</td>
<td>0.8976</td>
<td>0.4523</td>
<td>15</td>
</tr>
<tr>
<td>sample</td>
<td>0.9897</td>
<td>2.0</td>
<td>17</td>
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In case of the ageing experiment, the intensity values were normalized further to focus only to the spectral properties. Two significant ranges were found, where the change of
spectra was monotonous while the other parts remained the same. The first range was between 740 and 1040 nm, while the second one (1400 - 1500 nm) might refer to the moisture content since one of its significant absorption peaks is 1450 nm. (Figure 12.) The average intensity showed slow decrease by the 4-week ageing time. This change of the averages was negligibly smaller than the variance within groups. (Figure 13.)

**Figure 12.** Absorption spectra of the ageing experiment, average spectra in every week

**Figure 13.** Absorption spectra of vacuum-packed slices of beef sirloin aged for 4 weeks

PLS regression models were built by R Project algorithm to predict ageing time and location from spectral data. Using cross-validation and 17 factors the determinants were $R^2=0.9803$ for ageing time and $R^2=0.9762$ for predicting location. (Figure 14.)
6.2. Results of the Hyperspectral Inspection

After tracing some areas as Region of Interest (ROI) of pure flash and fat tissues, the meat and the fat classes on hyperspectral frames were segmented by applying Spectral Angle Mapper classification method. (Figure 15.)

The classification algorithm resulted the average reflected spectra of the different tissues, which were analyzed by R Project in a similar way to the instrumental measurements.
Even though the hyperspectral acquisition has generally much more noise, these reflected spectra were also enough to calibrate PLS regression models for ageing time prediction ($R^2=0.9857$, factors=15) and estimating the location of the slices ($R^2=0.9166$, factors=19). (Figure 16.) The statistical analysis also proved that the intensity was characteristic mostly to the location of the samples and less to the ageing time, while the ageing time caused the change of spectral properties.

7. Conclusion

Conventional spectroscopy and hyperspectral system can both efficiently determine the ageing time and the location of beef sirloin. These two properties can also be predicted properly by remote sensing even in noisy industrial environment and segmenting the different tissues of meat on multispectral images.

In case of the HSI, method of the measurements should be improved by avoiding the effect of uneven surface with measuring the samples under glass, and excluding mirroring with use of diffuse illumination.

On the base of hyperspectral measurement, classification and PLS regression model, the significant wavelengths of an inspected property can be calculated, those might be used by a non-contact multispectral measurement in an industrial application.

References

http://geol.hu/data/online_help/ApplyingSpectralAngleMapperClassification.html

Geesink http://www.sciencedirect.com/science/article/pii/S0309174002002693 - COR1,


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