AN APPROACH TOWARDS OVERCOMING THE PROBLEM OF REPRESENTATIVE SAMPLE SIZE WITH IMAGING SPECTROSCOPY

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Sampling methodologies are critical with respect to qualitative and quantitative assessment of inhomogenuous food products. Sampling schemes involving grinding, mixing, homogenization and extraction suffer inherently from two drawbacks, i.e. these methods are destructive and not readily applicable in an high-troughput industrial production area. An on-line quality-control measuring device will be able to measure relevant quality parameters from a representative sample area/surface at maximum production speed[1]. Ongoing developments in the fields of imaging spectroscopy and multivariate data analysis facilitate the use of objective on-line muscle food assessment systems. Foodprocessing equipment used for monitoring muscle food quality will increasingly be used in industrial environments, due to increasing demands from customers for product quality specifications.

We present an approach towards meeting the demands of on-line assessment of food quality characteristics. This preliminary study combines imaging techniques and spectroscopy and uses fast remote sensing of spectral information of large sample areas (3x7 cm), compared to the area (1x9 mm) of a solid sample holder of a research spectrofluorometer. The technique is demonstrated with experiments involving combined time-resolved image analysis, reflectance and fluorescence spectroscopy with the aim of measuring non-destructively early post-mortem events in minced salmon (Salmo salar), rainbow trout (Oncorhynchus mykiss) and trout fillets.

Princeton Instrument Imaging Spectrograph

The ST-138 system from Princeton Instruments Inc. is in combination with the Spectra Pro-150 spectrograph from Acton Research Corporation a flexible multi-use imaging and spectroscopic equipment. The system is based on the charge coupled device (CCD) sampling technique and grating diffraction light dispersion. An overview of the system is given in Figure 1 followed by a technical description of the system.

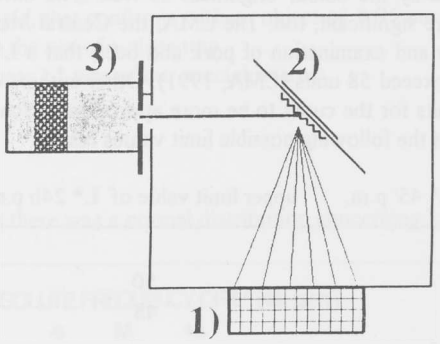


Figure 1. Simple schematic overview imaging spectrograph
1) liquid N_2 cooled CCD array. 2) Rotatable grating and mirror.
3) UV objective and slit width adjustment.

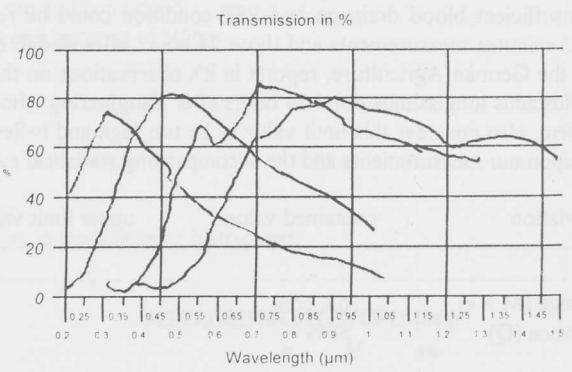
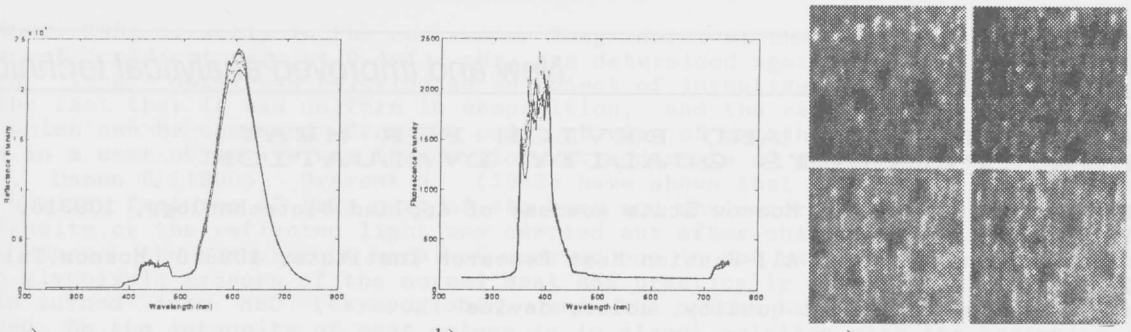


Figure 2. The grating characteristics for the four available gratings.

1) *Charge coupled device array*. The data sampling is performed using the CCD technique. The CCD array is of size 1100 (H) x 330 (V) pixels with the horizontal dimension giving the spectral resolution. The CCD array is UV-coated enabling sensitivity in the range 200-1100 nm (dependent on the grating). Furthermore, the CCD is back-illuminated (reducing signal loss due to absorption) and liquid nitrogen cooled resulting in a dark charge of below 0.0001 electrons/pixel-second at 153 K. The latter enables integration times of up to several hours without major dark current problems for basic research purposes.

2) *Spectrograph gratings*. The spectrograph enables spectral measurements of large surfaces using grating diffraction. Four rotatable gratings are available with different resolutions and wavelength characteristics providing an optimal spectral setup selection. The wavelength characteristics for the four available gratings are shown in Figure 2. By rotating the grating specific wavelength areas are focused on the CCD array and up to 330 spectra can be obtained in a few ms (depending on integration time). A fairly high reproducibility was found by recording 6 spectra (see Figure 3.) By rotating the grating holder to the mirror, total reflection is obtained. This is utilized to obtain a visual image of the object with a maximum slit width opening.

3) *UV-objective and slit width*. Using a UV-objective, it is possible to obtain the spectral and imaging information from a remote position. In the results presented here, the sample is approximately 1.10 m from the object. Selection of the slit width is a compromise between the measured sample and the obtained spectral resolution. Due to the inhomogeneity of meat, focus has been on representing a relatively large area of the sample (approx. 7x3 cm) on the cost of high spectral resolution. The multivariate data analysis does to some extent compensate for the drawback of this compromise when the measurements can be performed with a high reproducability.



a) b) c)

Figure 3. a) 6 *Reflectance spectra and* b) *Fluorescence spectra of salmon mince,* c) *images of salmon minces at 4 different post-mortem times: top left:t= 0 min., top right: t=120 min., bottom left t=270 min., bottom right t=420 min.*

Spectroscopic Measurements

The efficiency of the Princeton Instrument for analyzing inhomogeneous medias is demonstrated on a salmon study. During a period of 8 hours both fluorescence, reflectance and imaging data were acquired to follow spectrally the muscle development during aging. The reflectance was measured on a 7x3 cm surface from 300-700 nm (see Fig. 3). The fluorescence was measured with an excitation of 380 nm and the emission spectra were measured from 300-700 nm. The images were acquired every 30 min, and the total acquisition time was approximately 1 min/sample. Fresh (48 hours) iced salmon (class A, 5-10 kg) was filleted and minced and frozen in liquid nitrogen and kept in a freezer until gentle thawing in a refrigerator, immediately preceding the experiments. Fat content as measured with a $CHCl_3/CH_3OH$ extraction varied between 15,6-21,7 % (wet weight). Rainbrow trouts (5 kg) were caught 90 minutes before the start of the experiments.All measurements were carried out at 298 K. Multivariate analysis was carried out with program suites: Unscrambler (CAMO, Norway) and MATLAB (The Mathworks, USA).

Results

In the experiments both image anlysis, reflectance as well as fluorescence emission was measured as a function of time postmortem in order to study exploratively changes in quality parameters of raw mucle animal material. Table 1 gives the Root Mean Square Error of Prediction (RMSEP) and correlation coefficients (r) as calculated from Partial Least Squares Regression (PLSR) models, using optical data and mesuring times as datasets.[2]

	Stand. Reflection		Fluorescence[a]	
	RMSEP	r[c]	RMSEP[b]	r[c]
minced salmon	46	0.98	99	0.92
fresh trout	63	0.81	-	-
trout fillet	32	0.96	-	-

Table 1. Prediction errors based on experimental spectral data of muscle food samples over a range of 0-8 hours post-mortem.

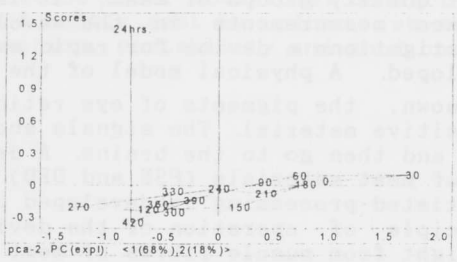


Figure 4. Principal Componant Analysis of reflectance spectra of minced salmon.

[a] Fluorescence emission was measured after excitation with light of 380 nm. [b] Root Mean Square Error of Prediction in minutes postmortem.[c] Correlation coefficient from Partial Least Squares regression with full cross validation.

The results indicate that a fast CCD-camera is capable of recording early post-mortem changes in muscle foods (see Figure 4). These changes in the muscle food during the measurement periods might relate to the rapid biological processes caused by the oxygen deprivation of the muscle tissue post-mortem and changes in fluorescent compounds of physiological importance, such as nicotine adenine dinucleotide hydride (NADH).[3] A fairly accurate prediction of the post-mortem time of a muscle food material will be useful in an industrial environment, if a generally valid calibration model could be established for many individuals from the same meat animal species. Our research efforts are directed towards remote spectral screening techniques for predicting mucle material freshness calibrated to changes in chemical composition and microbial and sensoric quality.

Conclusion

It has been shown that reflectance and fluorescence mesurements on a relatively large area (3x7 cm) of a muscle food sample can provide accurate information on the post mortem time. Both the rapid integration time as well as the integrating area makes this approach a suitable candidate for future on-line applications of quality monitoring in the muscle food industry.

[1] H.J. Swatland, 1995, On-Line Evaluation of Meat, Technomic, Lancaster, USA
[2] H. Martens and T. Næs, 1989, Multivariate Calibration, Wiley, Singapore
[3] L.Munck. (ed.), 1989, Fluorescence Analysis in Foods, Longman, NY, USA