CHAPTER 8

WARMED-OVER FLAVOUR IN PORCINE MEAT

- A COMBINED SPECTROSCOPIC, SENSORY AND CHEMOMETRIC STUDY

JESPER BRØNDUM[#,¤], DERREK V. BYRNE[#], LONE S. BAK[#,$], GRETE BERTELSEN[#] AND SØREN B. ENGELSEN[#*]

SUBMITTED TO MEAT SCIENCE

Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C

¤ SFK Technology, Transformervej 9, DK-2730 Herlev

$ Applied Food Technology, Biotechnological Institute, Holbergsvej 10, DK-6000 Kolding

* Author to whom correspondence should be addressed

ABSTRACT

The effectiveness of applying rapid spectral techniques in the prediction of meat quality in relation to pre-slaughter stress and Warmed-Over Flavour (WOF) was investigated. The present research investigated the relationship between pre-slaughter stress and WOF characteristics (after 0 to 5 days of storage) in porcine meat as evaluated by chemical, sensory and spectroscopic methods. Sensory evaluation, visual reflectance spectroscopy (VIS) and low-field ^{1}H NMR (LF-NMR) proved efficient in describing the different stress groups and the storage period, whereas fluorescence spectroscopy and the TBARS test were only able to follow the WOF storage period. Near Infrared Reflectance (NIR) and Raman scatter showed a very weak relationship to the pre-slaughter stress and WOF storage levels. Good correlation (up to r=0.93) of sensory terms was achieved with VIS and fluorescence spectroscopy and LF-NMR.

INTRODUCTION

Warmed-over flavour (WOF) development during chill storage of pre-cooked meat has been of continuing interest in recent years in relation to improving the quality of ready-to-eat meals and other convenience foods. It is generally accepted that the autoxidation of membrane phospholipids is largely responsible for the development of WOF (Ingene and Pearson, 1979; St. Angelo *et al.,* 1987). However, there is evidence to suggest that the degradation of proteins and heteroatomic compounds may also be implicated in the development of WOF (Spanier *et al.,* 1988; St Angelo *et al.,* 1988).

Several studies have been reported on the relationship between pre-slaughter stress and the quality of meat (Lewis *et al.,* 1989; Fernandez *et al.,* 1992; Watanabe *et al.,* 1996). However, investigations into the effects of pre-slaughter stress on WOF have not been reported until recent sensory work by Byrne *et al.,* (1999b). Pre-slaughter stress has been found to cause the depletion of muscle glycogen and therefore reduce post-mortem glycolysis resulting in meat with an increased ultimate pH (pH_u) (Warris *et al.,* 1989). Henckel *et al.,* (1998) have reported that pH decline post-mortem with increasing pre-slaughter stress level is similar in trend; however, the pH_u is increased. Chemical substances, such as epinephrine, injected pre-slaughter have also been found to result in increased pH_u (Sutherland and Cori, 1951). To produce an increase in pH_u epinephrine promotes glycogenolysis by enhancing phosphorylase and phosphofructokinase, both via cyclic AMP (Sutherland and Cori, 1951). Epinephrine has been proven to be a very efficient dose- and time-dependent modulator of pH decline (Fernandez *et al.,* 1992; Henckel *et al.,* 1998).

Spectroscopy is becoming a more and more attractive analytical technique for measuring quality parameters in food with decreasing instrument prices, improved equipment and improved data-analytical methods (chemometrics). The main advantages of using spectroscopic measurements are the rapid data acquisition, the possibilities for simultaneous determination of several quality parameters and the ability to replace expensive and slow reference techniques.

Fluorescence has demonstrated its use in several food quality applications such as meat composition (Jensen *et al.*, 1989), connective tissue (Swatland, 1995), wheat flour refinement (Jensen *et al.*, 1982), and sugar quality measurement (Munck *et al.,*1998). NIR has been used for qualitative and quantitative measurements since the 1950s (Benson, 1993). The applications of NIR to meat cover fat depths and fat softness (Swatland, 1995), fat, moisture, and protein contents (Isaksson *et al*., 1995) and most recently determination of water binding conditions (Forrest, 1997). Near infrared radiation represents the electromagnetic radiation at the wavelength range 800-2500 nm. The visual range is found at the slightly higher energy levels: 400-800 nm. A major obstacle in NIR measurements of food samples such as meat is the water

molecule which is among the best known NIR absorbers. The development of Fourier transform interferometers and NIR lasers (Hirschefeld and Chase, 1986; Porterfield and Campion, 1988) has made Raman spectroscopy an interesting and promising technique for use in the agricultural and food sciences. For example, FT-Raman has proved able to measure total level of unsaturation, cis/trans isomer ratios and the amount of free fatty acids in fats and oil (Sadeghi-Jorabchi *et al.* 1991; Ozaki *et al.* 1992; Engelsen, 1997). Raman spectroscopy is, unlike infrared spectroscopy, insensitive to the weakly polarisable vibrations of water and has therefore potential as a useful analytical technique for measuring heavily hydrated samples such as meat. LF-NMR is based on measuring the relaxation of the spins of hydrogen nuclei. By applying an external magnetic field the protons will align parallel and antiparallel to the field. A radio frequency (r.f.) pulse is used to rotate the spins 90 degrees after which the r.f. absorption of the nuclei is measured as they return to the equilibrium state and distinction between different proton media can be made. In the present application low-field (also called low-resolution) time-domain NMR is used as opposed to high-resolution frequency-domain NMR. LF-NMR has a long history of being used in the study of meat (Renou *et al.*, 1985; Fjelkner-Modig and Tornberg, 1986; Borisova and Oreshkin, 1992; Beauvallet and Renou, 1993) and is currently a standard method for determining the fat content in meat (Beauvallet and Renou, 1993).

These four spectroscopic techniques are applied with laboratory instrumentation. However, the measurements are non-invasive and non-contact and at least the optical spectroscopies NIR, fluorescence, and Raman might be further developed into on-line applications as have been observed in some other fields. LF-NMR could be advanced into an off-line system.

This present work investigates the relation between spectroscopic measurements and the sensory and chemical characterisation of the WOF meat. A wide range of spectroscopic techniques has been applied to the meat samples. Fluorescence emission spectroscopy, visual (VIS) and Near Infrared Reflectance (NIR) spectroscopy, Raman scatter spectroscopy and low-field (25 MHz) ^{1}H nuclear magnetic resonance (LF-NMR) have been used to measure the warmed-over flavour properties of the meat.

MATERIALS AND METHODS

MEAT SAMPLES AND PREPARATION

The Danish Institute of Agricultural Sciences, Foulum, Denmark provided pork muscles (*Musculus semimembranosus*). Four different sets of muscles were obtained (left and right side), each taken from a pig (4 female littermates) subjected to a

different stress level prior to slaughter (Table 1). Samples from each stress level were prepared, cooked and stored for 0, 1, 2, 3 and 5 days to facilitate WOF development, as previously described by Byrne *et al.* (1999a).

Spectroscopic measurements were performed on the cooked samples after homogenisation. Two replications were made on separate samples. Throughout the paper the samples are labelled according to the stress group (A, B, C, or D), followed by the number of days of storage (0, 1, 2, 3, or 5) and the reproduction number (1 or 4) (e.g. A34).

Table 1. Pre-slaughter stress treatments of the pigs

Code***	Stress Level
A*	Control, minimum stress prior to slaughter, pigs slaughtered immediately
B*	Pigs subjected to running (3.8 km/h) on a tread mill for 10 min prior to slaughter
C**	Pigs injected with 0.2 mg ephinephrine/kg live weight 15 h prior to slaughter
D**	Pigs injected with 0.3 mg ephinephrine/kg live weight 15 h prior to slaughter and 5 min running (3.8 km/h) on a tread mill immediately prior to slaughter

* 10 ml physiological saline solution (0.9 w/v) was injected subcutaneously in the neck region.

** ephinephrine was injected subcutaneously in the neck region.

*** patties from all cooking codes were stored for 0, 1, 2, 3 and 5 days to facilitate the WOF development.

SENSORY MEASUREMENTS

The data set from a sensory profile which used the stressed pork samples as previously described was averaged over ten assessors and four replicates, and used in the multivariate analyses of the present research (Byrne *et al.*, 1999b). The descriptive sensory vocabulary of 16 terms used to generate this sensory data set was developed by Byrne *et al.* (1999a) from an initial list of 40 terms using a panel of ten members (Table 3). A total of 7 daily sessions of approximately 2 hr each were required to obtain this list. A sample set, which covered stress level and WOF variation in the meat, was presented in the sensory laboratory for each of the vocabulary development sessions. Decisions on term removal leading to the final descriptive vocabulary were based on the fulfilment of selection criteria and were made via representative sample and reference assessment, panel discussions and interpretation of multivariate data analysis. The development of this descriptive vocabulary has been previously described in detail by Byrne *et al.* (1999a).

Table 3. *Statistics for the sensory and chemical reference data. SD is the standard deviation and N/A is non-applicable*

	Sensory Group	Mean	SD	Max	Min
Cardboard-like	Odor	5.98	0.59	7.05	4.84
Linseed oil-like	Odor	4.72	0.80	6.20	3.67
Rubber-like	Odor	5.31	0.65	6.52	4.07
Cooked pork meat-like	Flavour	6.76	1.02	8.65	5.26
Rancid	Flavour	6.16	0.92	8.07	4.51
Bread-like	Flavour	3.87	0.26	4.35	3.39
Vegetable oil-like	Flavour	3.35	0.28	3.83	2.83
Fish-like	Flavour	2.71	0.57	4.22	1.92
Nut-like	Flavour	2.88	0.28	3.36	2.32
Monosodium	Taste	5.53	0.76	6.58	4.28
Metallic	Taste	4.89	0.73	5.86	3.53
Bitter	Taste	4.33	0.44	5.24	3.77
Sweet	Taste	3.61	0.69	4.88	2.64
Salt	Taste	4.28	0.64	5.20	3.21
Sour	Taste	4.71	1.43	6.59	2.69
Astringent	Aftertaste	4.96	0.90	6.23	3.55
CD*	N/A	30.89	12.01	47.69	16.70
CD (meat)*	N/A	0.099	0.0038	0.014	0.0057
PC_2db*	N/A	34.49	3.47	39.51	29.61
PE_2db*	N/A	1.75	1.15	4.11	0.73
PC_1db*	N/A	42.50	6.25	51.23	34.40
PE_1db*	N/A	20.27	4.27	25.54	15.12
TBARS (mg/kg)	N/A	1.57	0.96	3.16	0.14
TBARS (m/kgdm)	N/A	4.81	2.99	10.29	0.42

* Only 8 samples (day 0 and day 5). CD: conjugated dienes (before and after (meat) extraction of lipids). PE: Phosphatidylethanolamin. PC: phosphatidylcholin. 1db: one double bond. 2db: two double bonds.

CHEMICAL MEASUREMENTS

Chemicals

Chloroform, methanol, 2-thiobarbituric acid, trichloroacetic acid and, cyclohexane, all of analytical grade, were purchased from E. Merck (D-64293 Darmstadt, Germany) and used without any further purification. Pyrogallol was purchased from Aldrich

Chemie (D-7924 Steinheim, Germany). Phosphatidylcholine and phosphatidyl-ethanolamine was purchased from Sigma.

Extraction of lipids

Meat (10.0 g) was homogenised in an Ultra Turrax with 100 ml chloroform:methanol (2:1 v/v). After homogenisation 25 ml 1mM $CaCl_2$ solution was added and the sample was mixed using an Ultra Turrax and centrifuged for 20 min at 1000 rpm. The chloroform phase was removed and the extraction procedure was repeated. The chloroform phase containing the extracted lipids was dried by vacuum evaporation. The 2×2 ml chloroform/methanol and 2 ml $CaCl_2$ were added to the dried sample and the sample was mixed and centrifuged for 20 min at 2500 rpm. The chloroform phase was removed and dried by vacuum evaporation.

Conjugated dienes

Extracted lipid (10 mg) was dissolved in 3 ml cyclohexan and the spectra were measured at 190 - 820 nm. The spectra were transformed to the secondary derivative, as described by Corongiu and Milia (1983) and the relative difference of absorbance at 254 nm and 238 nm was used to express the concentration of components with *trans, trans* conjugated bonds.

Separation of phospholipids

Lipid (10 mg) was dissolved in 100 or 200 µl chloroform and mixed carefully using a Vortex-mixer. 20 µl was injected in a HPLC with a diode array detector and a LiChrospher NH2-5, 25 cm x 4,6 mm id, LispNH-5-250A column at a column temperature of 45°C. A hexan/2-propanol/methanol/water (5:7:2:1, v/v) solution and a flow of 1ml/min was used as eluent. Phosphatidylethanolamin (PE) and phosphatidylcholin (PC) were measured at 203 nm using external standards. The fractions of PE and PC were collected individually and dried by vacuum evaporation.

Fatty acid composition in phospholipids

Fatty acid compositions in the isolated PC and PE fractions were analysed. The preparation and analysis of lipid methyl esters was performed according to Jart (1997). Fatty acid content of the phospholipids is expressed as percent of total fatty acid content

Thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was measured as TBARS by the extraction method described by Vyncke (1975) with a few modifications: Meat (10.0 g) from the surface of the patties was mixed with 30 ml of a 7,5 % trichloroacetic acid (TCA) solution with 0,1% propylgallate (PG) and 0,1% ethylenediaminetetraacetic acid, disodium salt (EDTA) for 45 s at 13500 rpm and filtered through a Whatmann filter no. 42. The extract was mixed with 0,02 M thiobarbituric acid (TBA), heated and cooled as described by

Vyncke (1975). The difference in absorbency, A_{532nm} - A_{600nm}, was measured on a spectrophotometer, where A_{600nm} was used to correct for sample turbidity.

Dry matter

Determinations of dry matter were conducted by drying 2.0 g meat from the surface of the patties in an oven at 104° C for 4 hours and weighing the sample after cooling for 15 min.

SPECTROSCOPIC TECHNIQUES

The samples were homogenised for the spectroscopic measurements in a coffee mill (Braun Type 4041, 150Watt). Following 10 seconds of homogenisation, the samples were placed in sample cups (except LF-NMR) equipped with a fluorescence-free quartz window with a diameter of 25mm. A compressible paper disk was used to force the meat against the quartz window. The samples were measured in randomised order and all measurements (except LF-NMR) were made at ambient temperature.

Visual and Near Infrared Reflection

Dispersive near infrared (NIR) data (including the visual region) were collected using a NIR Systems Inc. (Silver Spring, Maryland, USA) model 6500 spectrophotometer. The spectrophotometer uses a split detector system with a silicium (Si) detector between 400-1100 nm and a lead sulfide (PbS) detector from 1100 to 2500 nm. The angle of incident light was 180° and reflectance was measured at a 45° angle. The VIS/NIR reflection spectra were recorded using a spinning device for the sample cups described above and spectral data were converted to log(1/R) units. The spectra are divided into the VIS range (400-800 nm) and the NIR range (800-2500 nm).

Raman scattering

Near infrared Fourier transform Raman (Raman) analyses were carried out with a Perkin Elmer (Buckinghamshire, UK) System 2000 interferometer equipped with an Nd:YAG laser emitting at 1064 nm with a laser power of 1000 mW. Data were collected using an InGaAs detector and stored as Raman shifts between 3600 and ~300 cm^{-1}. The spectra were all recorded at 16 cm^{-1} resolution and averaged over 64 scans. The 180° back-scattering arrangement was used and no correction for the spectral response of the instrument was applied.

Fluorescence emission

Fluorescence data were collected on a Perkin Elmer (Buckinghamshire, UK) LS-50B grating spectrophotometer. The spectra were acquired by using a solid sample holder with a quartz window. A pulsed xenon lamp excites the sample, and the fluorescence signal is registered with a photomultiplier detector. A scan speed of 1500 nm/min and slitwidths of 8 nm were used on both excitation and emission. First complete

excitation-emission fluorescence landscapes were collected for two meat samples that were expected to cover the variation in the data material. Four excitation wavelengths: λ_{ex1}=280 nm, λ_{ex2}=330 nm, λ_{ex3}=370 nm, and λ_{ex4}=410 nm were selected from the landscapes. Emission spectra were recorded from the excitation wavelength plus 20 nm, to avoid the Rayleigh scattering, and up to 600 nm. In subsequent data treatment the four emission spectra for each sample were appended and stored as one spectrum (see Table **2a**).

Low Field ^1H Nuclear Magnetic Resonance

LF-NMR measurements were performed on a Maran Benchtop Pulsed NMR analyser (Resonance Instruments, Witney, UK), operating at a frequency of 23.2 MHz and equipped with an 18 mm variable temperature probe head. Transverse relaxation, T_2, was measured using the Carr-Purcell-Meiboom-Gill (CPMG) (Carr and Purcell, 1954; Meiboom and Gill, 1958) sequence, while longitudinal relaxation, T_1, was measured using the inversion recovery sequence (INVREC) (Vold *et al.*, 1968). NMR relaxation data were acquired as 16 scan repetitions for the CPMG pulse sequences and 4 scan repetitions for the INVREC pulse sequences using a two second relaxation delay between successive pulse scans (see Table 2b). The samples were equilibrated for 20 min. at the desired measurement temperature (26°C) and the sample probe temperature was kept constant by a continuous airflow. The meat samples were introduced into the NMR probe by placing the cylindrical samples (14 mm diameter, stamped out of the slices) into sealed glass tubes, which matched the inner diameter of the 18 mm NMR sample tubes.

Table 2a. Acquisition parameters for the spectroscopic measurements

	Fluorescence	VIS	NIR	Raman*
Instrument	Dispersive	Dispersive	Dispersive	FT
Sampling method	90° emission	45° reflection	45° reflection	180° scattering
Reference	None	Ceramic	Ceramic	None
X-variables	1501	200	850	3000
X-units	nm	nm	nm	cm^{-1}
X-min	300	400	800	300
X-max	600	800	2500	3600
Resolution	-	-	-	16
Accumulations.	-	16	16	64
X-sampling	0.5	2	2	4

* FT-Raman data were converted to frequency domain using medium Norton-Beer apodization. Fluorescence data collected using a slit width of 8 nm on both the excitation and emission side.

Table 2b. Important NMR pulse and acquisition parameters. Receiver delay is the time allowed for the magnetisation to recover before a subsequent pulse scan. Tau is the 90°-180° interpulse spacing in the CPMG sequence and the variable time delay between the initial 180° and the 90° pulses in the INVREC sequence

		CPMG*		INVREC	
Receiver delay (ms)	2000	Scans	8	SCANS	4
Receiver gain (%)	9.2	Tau (μs)	150	Tau (ms)	**
Dwell (μs)	0.5	Echoes	256	Points	16***
		Echo points	1/1		

* Only even echoes were collected
** Tau=[10,100,200,300,400,500,600,700,800,900,1000,1100,1200,1500,2000,4000]
*** Each point corresponds to the average of the first 4 points in the T_1-weighted free induction decay.

MULTIVARIATE DATA ANALYSIS

Variations in and correlations between spectral, sensory and chemical data are analysed using standard chemometric algorithms such as Principal Component Analysis (PCA) (Wold *et al.*, 1987) and Partial Least Squares regression (PLS) (Martens and Næs, 1993). These chemometric methods have the advantage of being able to deal with complex spectral information containing multivariate co-linear data (i.e. two neighbouring wavelengths show almost the same variation) and to allow for the data to be represented in a lower data dimension without loss of information.

Principal Component Analysis (PCA)

PCA is a fundamental method in chemometrics in which the original two-dimensional data matrix (*samples* × *variables*) is decomposed into a score matrix (*pc* × *samples*), a loading matrix (*pc* × *variables*) and a residual matrix (*samples* × *variables*). The systematic variation is described by a low number of principal components (PC1, PC2 etc.), each of which represents the outer product of scores and loadings. The loading vectors for the principal components can be considered as pure hidden profiles that are common to all the measured spectra. What makes the individual raw spectra different is the amounts (scores) of hidden profiles (Engelsen and Nørgaard, 1996).

Partial Least Squares Regression (PLS)

The standard chemometric calibration approach is to use Principal Component Regression (PCR) or PLS regression (Geladi and Kowalski, 1987). PLS is commonly used in quantitative spectroscopy, and consequently applied in this work, to correlate the spectroscopic data (rapid spectral measurements) with related sensory or chemical data (expensive, time consuming and laborious measurements). The model structure of PLS is the same bi-linear structure as in PCA where the independent variables are decomposed into a set of scores and the dependent variables are then regressed on these scores instead of the original variables. In the present study a specific version of the PLS algorithm, called PLS2 (Martens and Næs, 1993), is used. PLS2 enables the regression decomposition to be made with respect to several dependent variables simultaneously.

Prediction Validation

The number of PC's used in the regression models is crucial for the predictive performance of the PLS models and the optimal number of PC's is estimated during validation. In this study two true reproductions (different animals) of the 20 different samples were available. Consequently, the 40 measurements were split into a calibration set (reproduction 1) and a independent test (reproduction 4). Throughout the study the optimal number of PC's was determined in the calibration step by full cross (leave one out) validation and only true prediction results on the independent test set are reported (Wold, 1978). The predictive performance of the developed PLS models is evaluated and compared through the correlation coefficient and the parameter standard error of prediction (SEP). The sensory and the chemical reference values were averaged for the two reproductions and the same values were used for the two segments.

PCA and PLS analyses were carried out with the program suites: Unscrambler 7.0 (CAMO ASA, Trondheim, Norway) and MATLAB 5.2 (The Mathworks Inc., MA, USA).

RESULTS

SENSORY EVALUATIONS

The first two principal components (PC1 and PC2) from a PCA of the data for the16 sensory terms are shown as a score plot in Figure **1a**. PC1 tends to describe the variation in the stress level dimension in the samples. Across PC1 three groupings of the stress level samples are apparent with the scores for the A and B samples being confounded, while the C and D samples clearly separated. In PC2 the storage time of the WOF dimension is clearly depicted. In addition, the least WOF samples (day 0) are clearly separated from the most WOF samples (day 5). The progression of storage time is also apparent in PC2; however, the samples are not positioned similarly for each stress level. The loading plot of the PCA is shown in Figure **1b**. This shows how the sensory attributes are related to each other, e.g. the attribute Salt was strongly negatively correlated to Nut-like, as they were positioned opposite to each other in PC1. Overall, the space represented by PC1 and PC2 was widely spanned. This indicated that the vocabulary of 16 terms satisfactorily described the sensory dimensions in the scored sample set.

The scoreplot from the TBARS data is shown in Figure **1c**. Because the three TBA measures are highly correlated, almost the entire data variance is described in PC1. There is an interesting temporal development within PC1, with the samples from day 0 to the left and the samples from day 5 to the right. On day 0, the TBARS is not capable of separating the sample groups (A, B, C, and D). However, with increased oxidation time there is a clear indication that the sample groups become separated into three groups, with A and B samples in the first, C samples in the second, and D samples in the third. The C and D groups especially show a nearly linear tangent with time in the scoreplot, as indicated by the dotted lines.

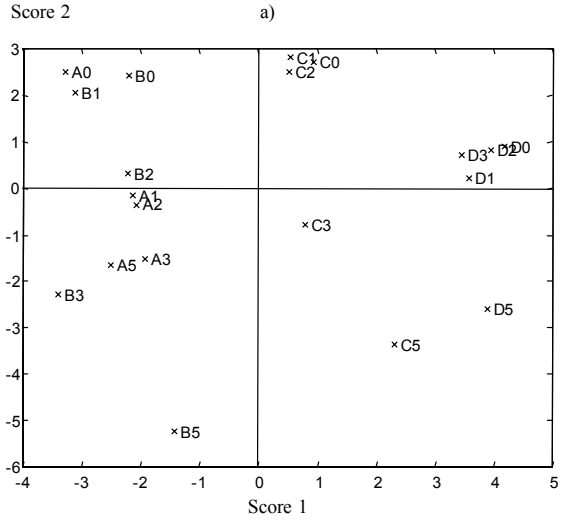

Figure 1. Continues.

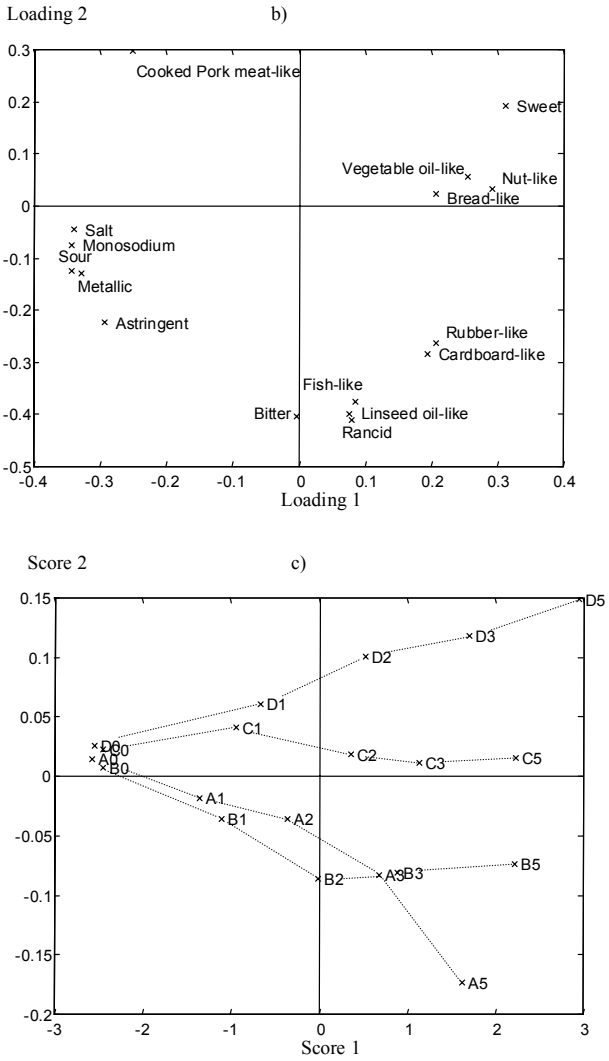

Figure 1. PCA overview of the sensory and chemical assessments of the WOF meat samples. a) Scoreplot from a PCA on the sensory data. b) Loadingplot from a PCA on the sensory data. c) Scoreplot from a PCA on the chemical data

VISUAL REFLECTION SPECTROSCOPY

The red pigment in muscles originates from myoglobin. When cooked porcine meat changes its colour from red to greyish-brown through complex reactions such as denaturation of myoglobin, Maillard reaction products, denatured globin nicotinamide hemichromes etc. (Swatland, 1989). Representative VIS spectra for 0, 1, 3, and 5 days of storage (sample A01) of the WOF meat samples are illustrated in Figure **2a,** from which three well-defined chromophores at 428, 546 and 654 nm can be identified. Scrutiny of the VIS spectra reveals a very high correlation (r=0.92) between the NIR log(1/R) intensities measured at 448 nm and 546 nm, indicating that the two peaks are

caused by the same effect, probably denatured deoxymyoglobin which in its native state has reported absorption maxima at 434nm (for the Soret band) and 555nm (Swatland, 1989). The chromophore at 654 nm may be due to denatured metmyoglobin for which in its native state has an absorption maximum at 630 nm (Bertelsen and Skibsted, 1987).

Figure **2b** shows a PCA scoreplot (PC1 versus PC2) based on the (2nd derivative) VIS spectra. PC1 and PC2 describe 76 % and 19 % of the variation in the spectra, respectively. It is noteworthy that the loading for PC2 holds the information from the 654 nm band which in turn is necessary for classifying the meat samples into WOF and stress levels.

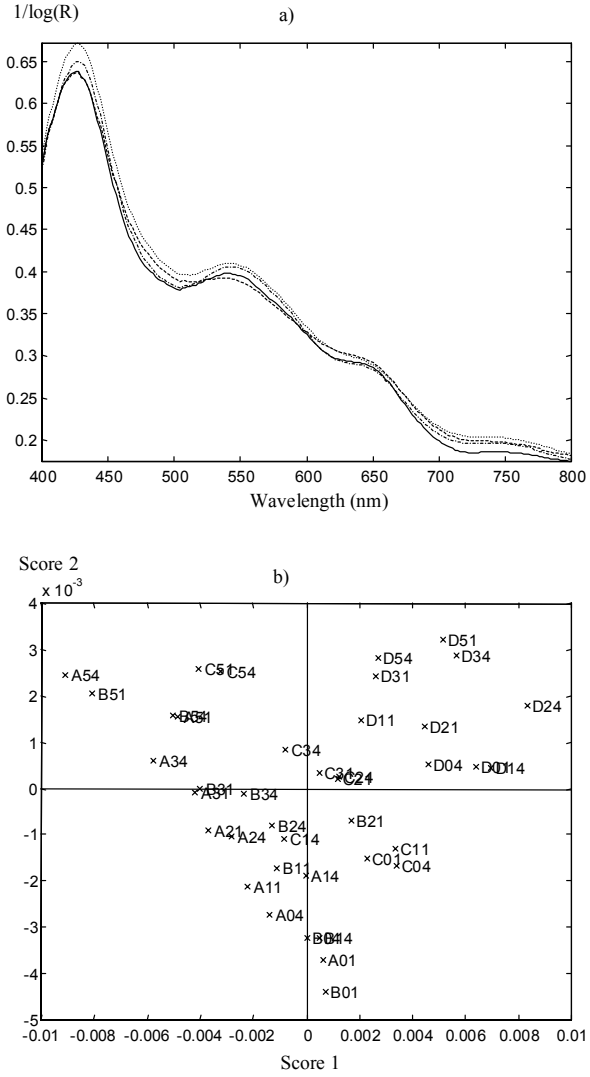

Figure 2. Visual reflectance spectroscopy of the WOF meat samples. a) VIS spectra of sample A01 at 0 (——), 1 (– – –), 3 (– · – ·), and 5 (-----) days storage. b) Scoreplot from a PCA on the (2nd derivative) VIS data.

The scoreplot is remarkable in that it divides the stress levels into three groups by a trend from the lower left quadrant to the higher right quadrant. A and B levels are completely overlapping while C and D are separated. The fact that A and B stress levels are collapsed is not surprising, as the B levels could not be distinguished from the reference A level by energy-metabolic factors (Henckel et al., 1999). With respect to the WOF levels (labelled 0 to 5 as the middle digit in the figure labels) the storage period is depicted by a trend from the lower right quadrant to the higher left quadrant. The reproductions (labelled 1 and 4 as the last item in the figure labels) are generally satisfactory. For some samples the reproduction is very accurate (as C21 and C24), whereas other meat samples are positioned relatively far apart in the scoreplot (as D11 and D14). The latter sample D14 is "misplaced" according to WOF level but not according to stress level.

The predictions of the sensory attributes from the VIS data listed in Table **4a** are generally very good. The VIS data predict at least one from each group observed in the scoreplot for the sensory terms (Figure **1a**) with high accuracy: Sweet is predicted with a correlation coefficient of r=0.93, Cooked Pork Meat-like with r=0.87, Linseed Oil-like with r=0.92 and Metallic with r=0.93.

The VIS spectra also displayed good correlations to the chemical reference measurements, see Table **4b**. Especially the TBA measurements are predicted well (r=0.95 and 0.96). The conjugated dienes and the phospholipids are also predicted well but the validity of the models are limited due to the low number of samples in both the calibration data set as well as in the validation set (8 in each).

Table 4b. PLS prediction results of the chemical attributes from fluorescence and VIS spectral ensembles. R is the correlation coefficient, Sep is the standard error of prediction and #PC is the number of PLS components needed for the actual model.

	VIS			Fluorescence		
	R	Sep	#PC	R	Sep	#PC
CD *	0.98	0.00075	3	0.87	0.0026	3
CD (meat)*	0.94	4.30	3	0.78	8.32	3
PC_2db*	0.64	0.88	3	0.70	0.90	3
PE_2db*	0.96	1.16	3	0.82	2.86	3
PC_1db*	0.45	3.21	3	0.55	3.10	3
PE_1db*	0.86	3.18	3	0.68	4.88	3
TBA (mg/kg)	0.96	0.28	3	0.64	0.75	3
TBA (m/kg dm)	0.95	0.94	3	0.59	2.42	3

* Only 8 samples are measured (4 stress levels at 0 an 5 days of storage)

NEAR INFRARED REFLECTION SPECTROSCOPY

Representative NIR spectra for 0, 1, 3, and 5 days of storage (sample A01) are shown in Figure **3**. As mentioned above the VIS and the NIR information is obtained with the same equipment, and the spectra are later split at 800 nm. The spectral shift at 1100 nm is caused by the detector shift. The NIR spectra of the meat samples are dominated by the water signal. The three broad peaks at 970 nm (O-H strech, second overtone), 1450 nm (O-H strech, first overtone) and 1940 nm (O-H strech + O-H deformation) are all due to water. In NIR spectra protein information is usually found around 1500 nm (N-H strech, first overtone) and above approx. 2000 nm (combination bands), but we were not able to find any such localised systematic variation in the NIR spectra of the meat samples. Scrutiny of the spectra revealed spectral variations around 1724 nm which can be related to methylene "fat" vibrations (C-H stretch first overtone) and, in fact, on basis of this single spectral variable it is possible to classify the D stress level. As a result of the of systematic information, the PCA performed on the NIR spectra is considerably less informative than the VIS PCA scoreplot with regard to interpretation of both the temporal oxidative development and the biological grouping. Only the D stress levels could be clearly classified (scoreplot not shown).

Results of the predictions of the sensory attributes from the NIR spectra are shown in Table **4a**. As apparent from the table, the predictions based on the NIR data are inferior to the VIS predictions. Only the Monosodium (r=0.94) and the Nut Like (r=0.71) attributes are slightly better predicted with the NIR data. In comparison with

the strong correlations between VIS spectra and the sensory attributes Sweet, Cooked Pork Meat-like, Linseed Oil-like and Metallic, the PLS predictions by NIR spectra are only slightly inferior, the Sweet parameter (r=0.8) being the worst case. The chemical reference analyses could not be predicted from the NIR spectra.

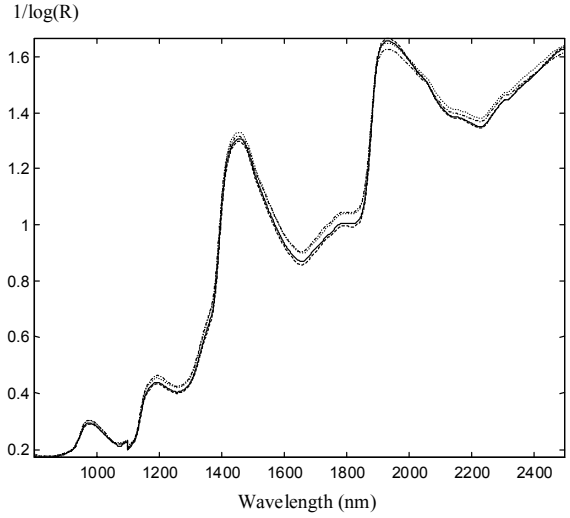

Figure 3. Near infrared reflectance spectroscopy of the WOF meat samples. NIR spectrum of sample A01 at 0 (——), 1 (– – –), 3 (– · – ·), and 5 (-----) days storage.

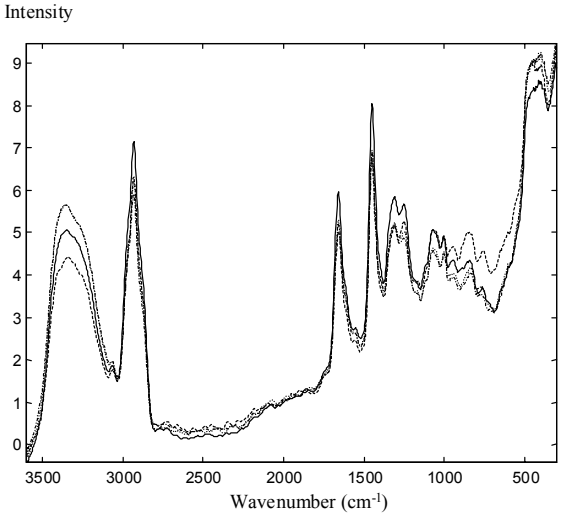

Figure 4. NIR FT-Raman stokes scatter of the WOF meat samples. Raman spectrum (Raman shifted) of sample A01 at 0 (——), 1 (– – –), 3 (– · – ·), and 5 (-----) days storage.

RAMAN SCATTERING

In theory Raman spectroscopy has some real advantages for the measurement of meat samples. Firstly, Raman signals can be transmitted through conventional optical fibres (quartz) and, secondly Raman is relatively insensitive to signals from water and thus

able to probe e.g secondary structure of proteins in solution. However, in our current set-up we suffered from the fact that the 1064 nm laser is too close to the third overtone of the O-H stretches which causes the information from aqueous compartments to almost vanish. Figure **4** shows the Raman spectra of sample A01 for 0, 1, 3, and 5 days of storage. The Raman spectrum includes a number of characteristic features that can be interpreted as follows: (1) A broad O-H and N-H stretching band with maximum at 3344 cm-1; (2) a small and sharp peak at 3064 cm-1 due to aromatic C-H stretches; (3) a very intense sharp and shouldered peak with maximum at 2934 cm-1 containing all the aliphatic C-H stretching information; (4) the amide I band with a peak maximum at 1663 cm-1 and the weak and broad amide II band at 1550 cm-1 indicating secondary amides. The amide I will also contain the aromatic C=C stretching vibrations which are relative intense in Raman. The strong and sharp peak at 1447 cm-1 is due to the symmetric bend of the methylene groups. (5) A sharp peak at 1001 cm-1 which is diagnostic for mono-, 1, 3- and 1, 3, 5-substituted phenylic compounds such as e.g. phenylalanine. Finally, the increasing background when going towards the Rayleigh line (lower Raman shift wavenumbers) is caused by fluorescence emission.

The results of the PCA with the Raman data resulted in practically no relation to the biological classes (data not shown). Also, the prediction of the reference data has been unsuccessful based upon the Raman spectra. Some of the sensory terms - Astringent (r=0.57), Metallic (r=0.65), Monosodium (r=0.57), Sour (r=0.56), Sweet (r=0.59), and Vegetable Oil (r=0.72) - can be predicted with some accuracy, but overall the results did not justify further analysis. We believe that this apparent lack of information in the Raman spectra is due to the fact that Raman spectroscopy is a technique with a relatively poor signal to noise ratio and the use of the 1064 nm laser. Future research should be directed towards Raman investigations of meat with e.g. 780 nm lasers which will be able to measure the high water content samples if background fluorescence is not detrimental.

FLUORESCENCE EMISSION

The four fluorescence emission spectra (excitation λ_{ex} of 280, 330, 370 and 410 nm, respectively) of sample A01 (0 days storage) are shown in Figure 5. Fluorescence emission peaks are observed at 350 nm for λ_{ex1}, at 390 nm and 440 nm for λ_{ex2}, at 440 nm for λ_{ex3}, and at 480 nm for λ_{ex4}. The 440 nm emission peak corresponds to the peak reported by Nakhost and Karel (1989) in oxidised beef meat and which was assigned to be due to the Schiff base structure. The emission peak observed at 390 nm has previously been reported by Jensen *et al.* (1989) when measuring fresh pork muscle. Jensen *et al.* (1989) also detected a peak at 460 nm in fresh fish muscle using an excitation wavelength of 366 nm which they assigned to the signal from the respiratory enzyme NADH. Stapelfeldt *et al.* (1992) assigned fluorescence peaks to

NADH and protein-tryptophane, but there is diverging considerations on whether the enzyme still exist in its native reduced form in the meat after cooking. For subsequent PCA only a region of 30 nm around each emission peak is used. The grouping of the stress groups is best described by the λ_{ex2} emission data, where the D samples are separated from the remaining, and the C samples are separated from the A and B samples with only a slight overlap (data not shown). The temporal and oxidative development is clearly depicted in the emission data from λ_{ex4}, which is shown in the scoreplot in Figure **5b**. The samples measured at 0 days storage after cooking are positioned to the right and the samples with 5 days of storage are positioned to the left. This indicates that NADH still exist in the meat samples.

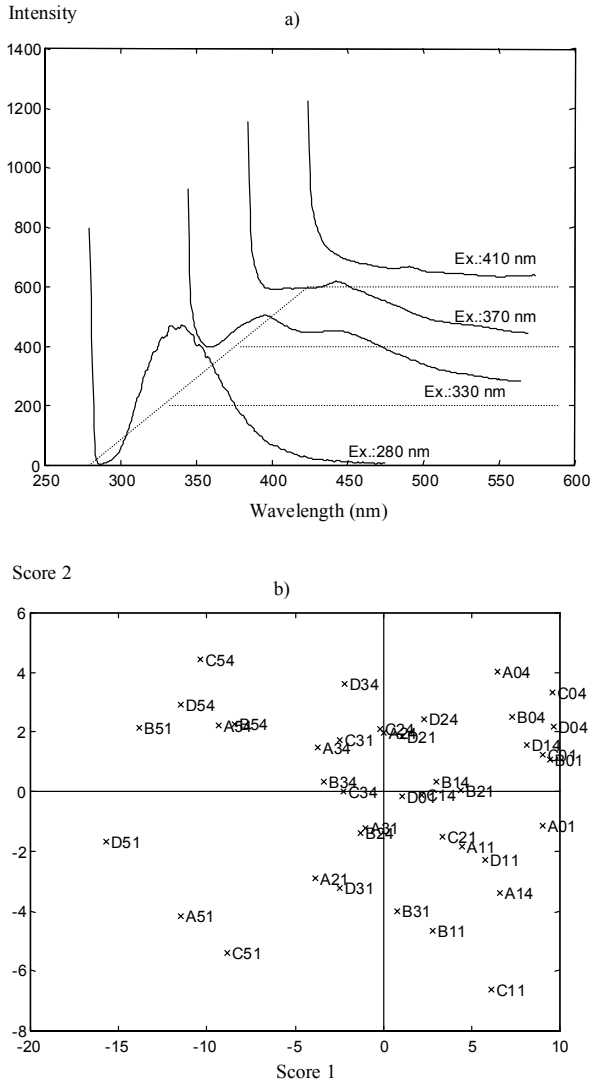

Figure 5. Fluorescence spectroscopy of the WOF meat samples. a) The four emission spectra measured for sample A01 using excitation wavelengths of 280 nm, 330 nm, 370 nm, and 410 nm. The horizontal dotted lines show the baseline of the three spectra. b) Scoreplot from a PCA on only the emission spectra recorded using excitation at 410 nm.

The PLS prediction results based on the fluorescence measurements are shown in Table **4a** and **4b** for the sensory, the chemical, and the physiological reference information. The results for the sensory parameters vary from r=0.26 (Bread-like) to r=0.80 (Linseed oil-like). In general the results are similar to the VIS predictions, but with lower correlations. Table **4b** presents the results of the test set validated fluorescence predictions of the chemical variables. The TBARS results are poorer than those obtained for the VIS data with r-values ranging from 0.59 to 0.64. The fluorescence information regarding the conjugated dienes and the PE/PC parameters (however with a reduced number of samples, Table 3) gives better results than TBARS – the visual information still being superior.

LOW-FIELD ^1H NUCLEAR MAGNETIC RESONANCE

LF-NMR relaxation curves are shown in Figure **6a** and **6b** for sample A01 for 0, 1, 3, and 5 days of storage. The INVREC (T_1) relaxation is shown in **6a** and the CPMG (T_2) relaxation is shown in **6b**. Low-field relaxation data is traditionally analysed using either ratio determination using two well chosen relaxation times or through multi-exponential curve fitting (Renou et al., 1985; Fjelkner-Modig and Tornberg, 1986; Borisova and Oreshkin, 1992). In this work, however, we have analysed the relaxation curves using multivariate data analysis, taking advantage of the entire data material. In a simple PLS application on fish muscles, this approach, utilising the so-called first order advantage, has shown to improve the accuracy of the PLS predictions with not less than 20 percent (Jepsen et al., 1999; Bechman et al., 1999).

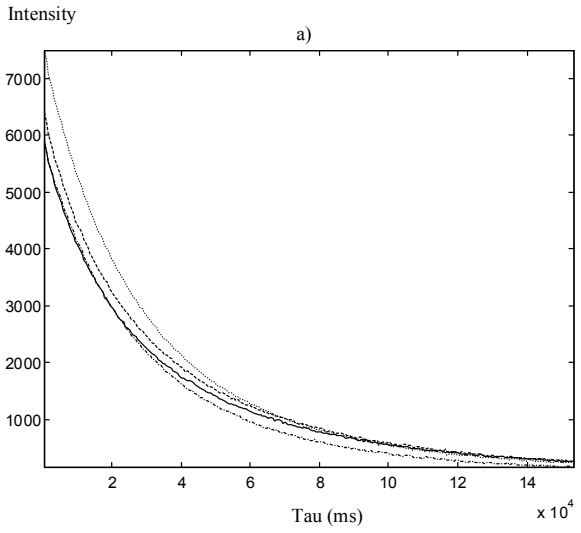

Figure 6. Continues.

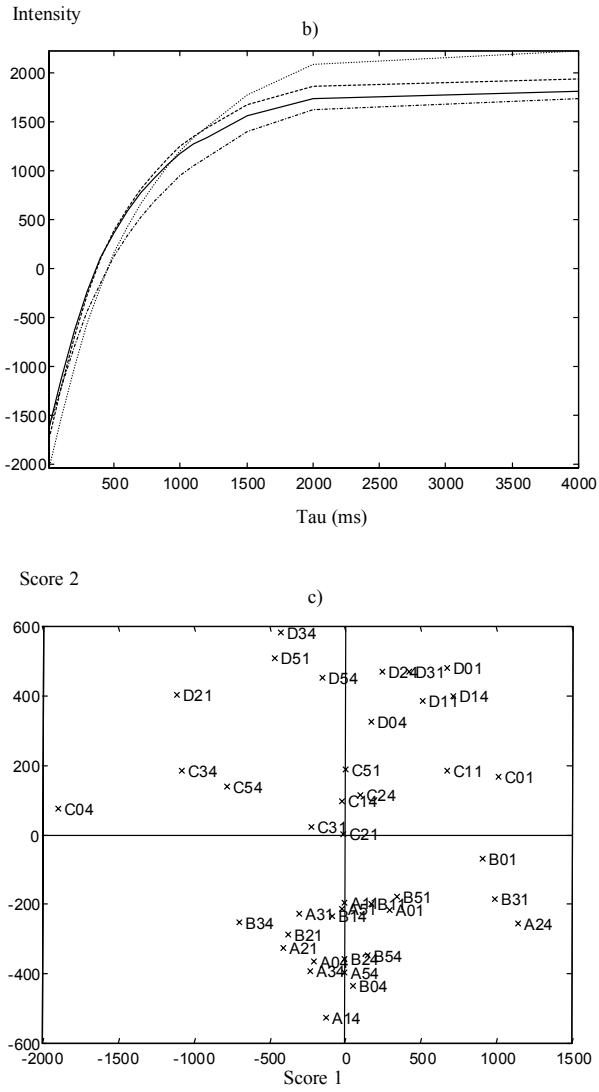

Figure 6. Low-field NMR relaxation of the WOF meat sample A01 at 0 (——), 1 (– – –), 3 (– · – ·), and 5 (-----) days storage. a) CPMG (T_2) relaxation. b) INVREC (T_1) relaxation. c) Scoreplot from a PCA on the INVREC relaxation data.

Figure **6c** shows the scoreplot of the first two PC's from the PCA on the INVREC LF-NMR measurement. Almost all the variance in the data is explained by these two components (78% + 22%). Similar to the results obtained for the sensory and the VIS reflectance measurements, there is a good separation of the C and D stress levels defined in Table **1**. The A and B stress levels are confounded as also observed for the sensory and VIS data. Inspection of the stress group D alone shows a good indication of the storage labels from right to left, although not quite as clear as for the sensory and VIS data. A similar indication for the C group is indicated (although C04 appears to be an outlier), but the tendency is not observed from the A and B group. The

reproduction of the measurement of the same sample is generally not very impressive. Some samples are well reproduced (as e.g. D11 and D14), whereas others (as e.g. D21 and D24) are located far apart in the scoreplot. The grouping tendencies for the PCA on the CPMG sequence (not shown) are less structured than those observed for the INVREC data.

LF-NMR relaxation data have previously been reported to be highly correlated to different sensory attributes of porcine muscles (Fjelkner-Modig and Tornberg, 1986) and Table **4a** shows the PLS prediction results of the sensory attributes based on the LF-NMR INVREC and CPMG relaxation data. The information held by the two different relaxation mechanisms give rise to a quite different prediction performance for the 16 sensory attributes. Where parameters like Astringent, Monosodium, Salt, and Sour all are predicted with correlation coefficients higher than 0.84 with the INVREC relaxation data, other attributes such as Bitter, Fish Like and Rancid cannot be predicted. Inspecting only those sensory parameters with correlation coefficients above 0.7 (7 of the 16 variables), then the performance of the INVREC measurements is the better of the two techniques for 6 of the 7 parameters. Yet, the difference between the performance of the two techniques is not dramatic, and considering the much more rapid sampling time for the CPMG technique, it is definitely worth considering continuing the work with this technique. The good predictions of the Salt term is very interesting, due to an expected indirect relation between the salt concentration and water mobility and binding.

DISCUSSION

The sensory vocabulary, the VIS reflectance and the LF-NMR relaxation were all able to classify the four stress groups into three categories, AB, C and D. Separation of A (control) and B (exercise) samples was not possible. This is in agreement with the results reported by Henckel *et al*. (1999) who found no significant difference in the ultimate pH values in the raw meat of the A and B groups of the same meat material. The three techniques represent very different information in the samples and the similarity of the sample characterisation is therefore very interesting. The information in the sensory data that was able to separate the samples into the different stress levels originated mainly from the terms Sweet, Nut-like, Bread-like and Vegetable Oil-like versus Salt, Sour, Monosodium and Metallic. These two term groupings separated the stress levels across PC1 of the sensory PCA (Figure 1b).

The VIS information originates from a delicate balance of chromophores such as heme groups in different environments and Maillard reaction products and will also reflect changes in the optical properties caused by the difference in texture of the three groups. The LF-NMR information which is dominated by the water signal is caused

by differences in the compartmentalisation of the water (intracellular/extracellular) and/or by differences in the water structure (structured water/bulk water) (Tornberg et al., 1999). It is interesting to note that LF-NMR gives good correlations to salty flavours as the salt concentration will affect the mobility of the water (water binding) in the meat and because the salt concentration should be correlated to oxidation. Moreover, the fact that LF-NMR is able to distinguish between pre-slaughter stress levels after cooking and storage is considered an important result, as it strongly suggests that the physical structure of the meat is significantly affected by stressing and that LF-NMR has the potential to be a powerful tool in the control of meat quality.

That the NIR and Raman spectral information did not separate the samples very well is mainly caused by the detrimental influence of water on the two chosen techniques.

The different WOF storage levels were also detected with the sensory evaluation, the VIS reflectance and the LF-NMR relaxation. The separation in WOF samples for the different stress levels was explained mainly by the terms in PC2. The sensory panel scores which formed the basis of this differentiation across PC2 were Sweet and Cooked pork meat-like versus the oxidation terms Rancid, Linseed oil-like, Cardboard-like and Rubber-like. (Figure1b). The information obtained by VIS is believed to be due to effects in the meat which result from oxidation reactions which affect meat proteins e.g. denaturation of myoglobin. The results reported are similar to the observations made by Zhu and Brewer (1998) who followed fresh PSE, DFD, and normal porcine muscle from the 0 to 7 days of storage with colour measurements. They found the a* value to decrease significantly after 4 days of storage due to the conversion of oxymyoglobin to metmyoglobin.

The absent NIR and Raman information on the WOF storage levels indicated that in addition to the water problem mentioned previously, the concentration of oxidation products was below the detection limit of these less sensitive methods. Furthermore, it was expected that the WOF dimension in the meat samples would be observed by changes in fluorescence spectra. However, no such systematic information was found.

The TBA test on the other hand did register the oxidation of the samples, and we must therefore conclude that the fluorophores present in the meat samples (protein-tryptophane and NADH) were not affected by the oxidative changes.

The prediction of the WOF was especially successful with the VIS reflectance. For the sensory terms, the VIS measurements had the highest correlations for 11 of the 16 descriptive terms. NIR and LF-NMR each achieved highest correlation for 2 of the 16 terms, whereas fluorescence only outperformed the other techniques for a single sensory term. For all the spectroscopic techniques, low correlations were generally observed for the sensory terms with low variation (e.g. Bread-like, Nut-like, and Vegetable oil-like). It is important to notice that all the spectroscopic techniques

(except Raman) predicted at least one of the sensory terms from each of the four groups observed in different regions of the loading plot in Figure **1b**. In terms of future detailed studies of meat quality, where sensory assessments may be limited due to their costly nature, it was encouraging to observe the very high spectroscopic correlations to a majority of the sensory attributes. Therefore, spectroscopic methods could be used as an inexpensive rapid alternative in the prediction of the sensory quality of cooked meats.

CONCLUSION

An experiment that utilised spectroscopy (LF-^{1}H NMR, VIS reflectance, near infrared reflectance, Raman scattering, and fluorescence emission), sensory descriptive profiling (16 terms) and chemical measurements (TBA, PE, PC, and CD) in the assessment of WOF in meat from animals with different levels of pre-slaughter stress levels were performed. The data from the various techniques were interpreted separately for each technique and through multivariate data analytical techniques, together to determine the predictability that existed between the different data sets. Prior to slaughter the animals were subjected to four different stress treatments, which after meat processing and development of WOF in cooked samples could be separated into three groups by the sensory descriptive terms, the VIS reflectance and the LF-^{1}H NMR. The same techniques were also capable of following the storage time over which WOF developed. TBA also followed the development with storage time, and after 3 days of storage grouping into the three stress level groups was observed. Neither fluorescence or Raman scattering were successful in separating the stress groups or the following WOF development. The spectroscopic methods were also used for predicting the sensory and the chemical quality parameters. In general, the VIS reflectance was superior to the other techniques in predicting the sensory terms, but LF-NMR and fluorescence were also successful in the predictions. In general, VIS reflectance and LF-NMR proved to be the most optimal techniques for probing WOF development in meat. The two techniques complemented each other well, but if one is to be selected VIS reflectance has some obvious advantages in the measurement acquisition time and the sampling technique. For the VIS reflectance sampling was performed with rotating sample cups, but this could easily be modified to a more readily applicable technique using e.g. fibre optics. On the other hand, LF-NMR may have an advantage in measuring the entire volume of the sample which will reduce influence of surface effects. It is also to be expected that NMR will be less sensitive to smaller differences in the meat structure caused by different animals, handling procedures etc. and therefore would be easier to calibrate more globally.

Based on spectroscopy's extremely good predictive ability of sensory quality in the samples it has the potential for use in a rapid on-line industrial situation to predict the sensory quality of cooked meat samples.

ACKNOWLEDGEMENTS

The authors wish to thank the Danish Academy for Technical Sciences (ATV), The Danish Centre for Advanced Food Studies (LMC), the Danish Research Councils (SJVF), EU-contract (FAIR975003) and EU AFFLUENCE programme (AIR-2-CT94-1416) for financial support. The Danish Institute of Agricultural Sciences, Foulum, is gratefully acknowledged for supplying the meat samples.

REFERENCES

Beauvallet, C. and Renou, J. (1993) Applications of NMR in Meat. *Trends in Food Science & Technology* **3**, 241-246.

Bechmann, I.E., Pedersen, H.T., Nørgaard, L. and Engelsen, S.B. (1999) Comparative Chemometric Analysis of Transverse Low-Field ^{1}H NMR relaxation Data, *Advances in Magnetic Resonance*, submitted.

Benson, I.B. (1993) Compositional Analysis Using Near Infrared Absorption Analysis. In: *Instrumentation and Sensors for the Food Industry*, Kress-Rogers, E. (Ed), Butterworth-Heinemann Ltd. pp 121-166.

Bertelsen, G. and Skibsted, L.H. (1987) Photooxidation of Oxymyoglobin. Wavelength Dependence of Quantum Yields in Relation to Light Discoloration of Meat, *Meat Scie*nce **19**, 243-251.

Borisova, M. A. and Oreshkin, E. F. (1992) On the water condition in pork meat. *Meat Science* **31**, 257.

Byrne, D.V., Bak, L.S., Bredie, W.L.P., Bertelsen, G. and Martens, M. (1999a) Development of a sensory vocabulary for warmed-over flavor: part I. in porcine meat. *Journal of Sensory Studies* 14, 47-65.

Byrne, D.V. Bredie, W.L.P. and Martens, M (1999b) Sensory profiling of cooked stored and reheated porcine meat patties. *Journal of Food Science*. In preperation.

Carr, H. and Purcell, E. M. (1954) Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Physical Reviews* **94**, 630-638.

Corongiu, F.P. and Milia, A. (1983), An improved and simple method for determining diene conjugation in autoxidized polyunsaturated fatty acids. *Chem. Biol. Interactions* **44**, 289-297.

Engelsen, S.B. (1997) Explorative Spectrometric Evaluations of Frying Oil Deterioration. *Journal of American Oil Chemists' Society* **74**, 1495-1508.

Engelsen, S.B. and Nørgaard, L. (1996) Determination of Quality Parameters in Amidated Pectins as Evaluated by Chemometrics. *Carbohydrate Polymers* **30**, 9-24.

Fernandez, X., Forsild, A., Mågård, M., Möller, M. and Tornberg, E. (1992). Effect of time between adrenaline injection and slaughter on the rate and extent of post-mortem metabolism in porcine skeletal muscle. *Meat Science* **31**, 287-298.

Fjelkner-Modig, S. and Tornberg, E. (1986) Water distribution in Porcine *M. longissimus dorsi* in relation to Sensory properties. *Meat Science* **17**, 213-231.

Forrest, J. C., Sheiss, E. B., Morgan, M., and Gerrard, D. E. (1997). Pork Quality Measurement Tools - Now and in the Future. *NPPC Pork Quality Summit Proc., Des Moines, IA* , 79-96.

Geladi, P. and Kowalski, B. R. (1987) Partial Least-Squares Regression: A Tutorial. *Analytica Chimica Acta* **185**, 1-17.

Henckel, P., Karlsson, A., Oksbjerg, N. and Petersen, J.S. (1999) Standardisation of Post Mortem pH-Decrease in Pig Muscles, submitted.

Hirschefeld, T. and Chase, B. (1986) FT-Raman Spectroscopy: Development and Justification. *Applied Spectroscopy* **40**, 133-137.

Ingene, J.O. and Pearson, A.M. (1979). Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *J. Food Science*, **44**, 1285-1290.

Isaksson, T., Tøgersen, G., Iversen, A. and Hildrum, K.I. (1995) Non-destructive determination of fat, moisture and protein in salmon fillets by use of near-infrared diffuse spectroscopy. *Journal of the Science of Food and Agriculture* **69**, 95-100.

Jart, A. (1997) The magnetic Field as an Additional Selectivity Parameter in Fat Hydrogenation. *Journal of the association of oil chemists society* **74**, 615-617.

Jensen, S.A., Munck, L. and Martens, H. (1982) Botanical constituents of wheat and wheat and wheat milling fractions. I. Quantification by autofluorescence. *Cereal Chemistry* **59**(6), 477-484.

Jensen, S. A., Reenberg, S. and Munck, L. (1989) Fluorescence analysis in fish and meat technology. In: *Fluorescence Analysis in Foods*, Munck, L. (Ed.), Longman, Harlow, UK, 181-192.

Jepsen, S.M., Pedersen, H.T. and Engelsen, S.B. (1999) Application of Chemometrics to Low-Field ^{1}H NMR Relaxation Data of Intact Fish Flesh, *Journal of the Science of Food and Agriculture*, submitted.

Lewis, P.K.Jr., Rakes, L.Y., Brown, C.J. and Noland, P.R. (1989). Effect of exercise and pre-slaughter stress on pork muscle characteristics. *Meat Science* **26**, 121-129.

Martens, H. and Næs, T. (1993) Multivariate Calibration, Wiley, New York.

Meiboom, S. and Gill, D. (1958) Modified spin-echo method for measuring nuclear relaxation times. *The Review of Scientific Instruments* **29**, 688-691.

Munck, L., Nørgaard, L., Engelsen, S., Bro, R. and Anderson, C. A. (1998) Chemometrics in food science - a demonstration of the feasibility of a highly exploratory, inductive evaluation strategy of fundamental scientific significance. *Chemometrics and Intelligent Laboratory Systems* **44**, 31-60.

Nakhost, Z. and Karel, M. (1989) Fluorescence due to interactions of oxidizing lipids and proteins in meats. In: *Fluorescence Analysis in Foods*, Munck, L. (Ed.), Longman, Harlow, UK, 193-214.

Ozaki, Y., Cho, R., Ikegaya, K., Muraishi, S. and Kawauchi, K. (1992) Potential of Near-infrared Fourier Transform Raman Spectroscopy in Food Analysis. *Applied Spectroscopy* **46**, 1503-1507.

Porterfield, Dr. and Campion, A (1988) Fluorescence-free scanning Raman spectroscopy, *J.Am.Chem.Soc.* ***110****, 408-410.*

Renou, J.P., Kopp, J. and Valin, C. (1985) Use of low resolution NMR for determining oil content in meat products. *Journal of Food Technology* **20**, 23-29.

Sadeghi-Jorabchi, H., Wilson, R. H., Belton, P. S., Edwards-Webb, J. D. and Coxon, D. T. (1991) Quantitative analysis of oils and fats by Fourier transform Raman spectroscopy. *Spectrochimica Acta* **47**(A), 1449-1458.

Spanier, A.M., Vincent Edwards, J. and Dupuy, H.P. 1988. The warmed-over flavor process in beef: a study of meat proteins and peptides. *Food Technol.* **46**, 110-118.

St. Angelo, A.J., Vercellotti, J.R., Legendre, M.G., Vinnett, C.H., Kuan, J.W., James, C. and Dupuy, H.P. (1987) Chemical and Instrumental Analyses of Warmed-Over Flavor in Beef, *Journal of Food Science* **52**(5), 1163-1168.

St. Angelo, A.J., Vercellotti, J.R., Dupuy, H.P. and James, C. (1988) Assessment of beef flavor quality: A multidisciplinary approach, *Food Technology* **42**(6), 133-138.

Stapelfeldt, H., Bjørn, H., Skovgaard, I.M., Skibsted, L.H., Bertelsen, G. (1992). Warmed-over Flavour in Cooked Sliced Beef. *Z. Lebensm. Unters. Forsch.* 195, pp. 203-208.

Sutherland, E.W. and Cori, C.F. (1951). Effect of hyperglycemic-glycogenolytic factor and adrenaline on liver phosphorylase. *J. Biol. Chem.* **188**, 531-543.

Swatland, H.J. (1989) A Review of Meat Spectrophotometry (300 to 800 nm*), Journal of the Canadian Institute of Food Science and Technology* **22**(4), 390-402.

Tornberg, E., Wahlgren, M., Brøndum, J. and Engelsen, S.B. (1999), Instrumental analysis of pre-rigor conditions in beef at varying temperature- and pH-falls, submitted.

Vold, R. L., Waugh, J. S., Klein, M. P., and Phelps, D. E. (1968). Measurement of Spin Relaxation in Complex Systems. *Journal of Chemical Physics* **48**, 3831-3832.

Vynche, W. (1975) Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (*Scomber scombus L.*) *Fette, Seifen, Anstrichmittel* **77**, 239-240.

Warris, P.D., Bevis, E.A. and Ekins, P.J. (1989). The relationships between glycogen stores and muscle ultimate ph in commercially slaughtered pigs. Br. Vet. J., *145*, 378-383.

Watanabe, A., Daly, C.C. and Devine, C.E. (1996). The effects of the ultimate pH of meat on tenderness changes during ageing. *Meat Science* **42**, 67-78.

Wold, S. (1978) Cross-validatory estimation of the number of components in factor and principal components models. *Technometrics* **20**, 397-405.

Wold, S., Esbensen, K. and Geladi, P. (1987) Principal Component Analysis. *Chemometrics and Intelligent Laboratory Systems* **2**, 37-52.

Zhu, L.G. and Brewer, M.S. (1998) Discoloration of Fresh Pork as Related to Muscle and Display Conditions. *Journal of Food Science* **63**(5), 763-767.