JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Monitoring Chemical Changes of Dry-Cured Parma Ham during Processing by Surface Autofluorescence Spectroscopy

JENS K. S. MØLLER,*,† GIOVANNI PAROLARI,‡ LAURA GABBA,§ JAKOB CHRISTENSEN,# AND LEIF H. SKIBSTED†

Food Chemistry and Food Technology, Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark; Stazione Sperimentale per l'Industria delle Conserve Alimentari, V.le F. Tanara 31/A, 43100 Parma, Italy; and Centro Ricerche Produzioni Animali, 42100 Reggio Emilia, Italy

Parma hams at various processing stages were investigated by surface autofluorescence spectroscopy. Fluorescence "landscapes" of raw meat and salted (3 months), matured (11 and 12 months), and aged (15 and 18 months) Parma hams were obtained, and a three-dimensional data array (sample × emission × excitation) was used to develop a PARAFAC model including five components, which all exhibited characteristics of pure fluorophores regarding both excitation and emission spectra. The relative amount of each component related strongly to the processing stage, and sample age showed good correlation to fluorescence data ($R = 0.98$), with a relative error of prediction of approximately 1 month. Fluorescence measurements from samples of either semimembranosus or biceps femoris were used to predict chemical or sensory reference data, yielding good correlation for biceps femoris data, thereby enabling moisture content, sensory and instrumental color, and proteolysis value to be fairly well predicted. Overall, surface autofluorescence of Parma hams proved to hold relevant information, relating to major chemical/physical changes during processing. It is concluded that fluorescence spectroscopy has potential as an innovative method of quality control in dry-cured ham.

KEYWORDS: Dry-curing; ham; process control; fluorescence landscapes; chemometrics

INTRODUCTION

Dry-cured Parma ham is a traditional meat product manufactured according to an additive-free long-term maturing process, requiring more than 12 months of aging for the characteristic flavor to be fully developed. Although most steps in production have been automated, the process control still relies on traditional evaluation methods. For this reason there is interest in the application of instrumental predictive methods relevant to quality assessment at several manufacturing stages.

A number of important parameters pertaining to the quality of the raw material or dried hams at intermediate stages of processing have been identified, including the pH of raw meat (*1*), NaCl concentration (*2*), and, more recently, the endogenous proteolytic activity during maturation (*3*, *4*). In addition, a variety of quality defects of either microbial or chemical origin have been identified (*5*). Among the latter class of defects are formation of white tyrosine crystals on the surface, excessive softness, and bitter taste (*3*). These defects are related to abnormal protein breakdown, and efforts are being made to predict proteolysis in dried ham from raw meat analysis (*6*). In light of the long processing time and for standardization purposes, it is crucial to identify defective hams as early as possible.

To improve the quality control in dry-cured ham production, there has been a demand for innovative and fast methods for assessing quality during processing (*7*). Several studies have shown that fluorescence spectral data from beef correlate with sensory overall toughness (*8*), connective tissue content (*9*, *10*), and Warner−Bratzler shear value (*11*). Furthermore, the degree of lipid oxidation in chicken meat has also been evaluated by autofluorescence spectroscopy (*12*). Currently, instruments that are suitable for on-/at-line measurements of autofluorescence either at the sample surface or using an insertion probe are commercially available (*9*, *10*), and an advantage of fluorescence spectroscopy over traditional methods used in the meat processing industry, apart from its speed, is the nondestructive nature of the measurement. Given the significant physical and chemical changes occurring during the process of dry-curing, fluorescence spectroscopy may be an appropriate method for monitoring quality or maturation degree while the process is in progress. Multivariate techniques have been evaluated in the analysis of meat quality (*13*), and meat products like most other food products are of a complex nature, which calls for explorative

* Author to whom correspondence should be addressed. Tel.: +45 2835 3290. Fax: +45 2835 3344. E-mail: jemo@kvl.dk.
† Food Chemistry, Department of Dairy and Food Science, The Royal Veterinary and Agricultural University.
‡ Stazione Sperimentale per l'Industria delle Conserve Alimentari.
§ Centro Richerche Produzioni Animali.
# Food Technology, Department of Dairy and Food Science, The Royal Veterinary and Agricultural University.

10.1021/jf025662h CCC: $25.00
Published on Web 01/24/2003

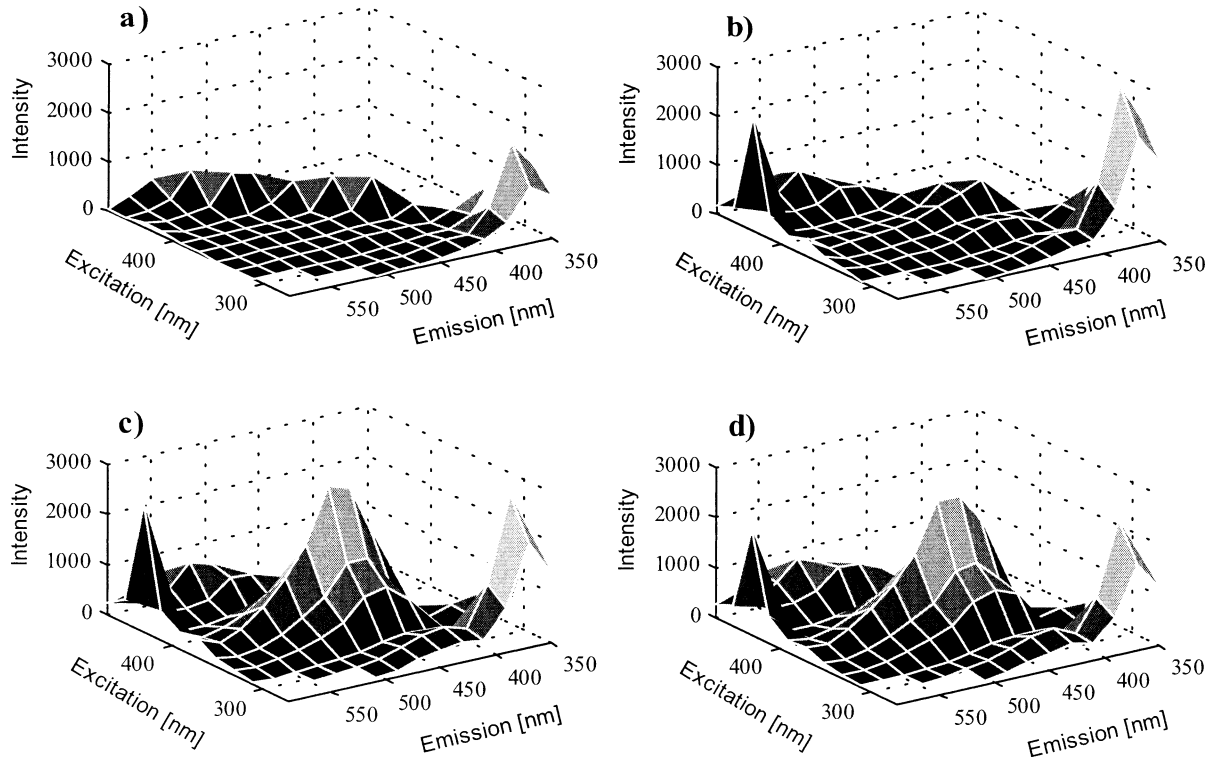

Figure 1. Representative reduced fluorescence landscapes (13 × 11) of raw meat (a), salted Parma ham (b), 11 month matured Parma ham (c), and fully matured Parma ham (d). Landscapes shown represent data used in the PARAFAC model.

analysis methods. The combination of fluorescence and chemometrics, including multiway methods appropriate with fluorescence spectral data (*14*), can prove to be a strong tool in the processing control of dry-cured hams, enabling the identification and quantification of pure fluorescence components that may correlate with maturation degree and quality. A previous attempt to use multivariate methods with sensory and instrumental analysis of dry-cured ham led to good relationships for sensory firmness and taste, but not for tristimulus color (*7*).

The main objective of the present study was to evaluate surface autofluorescence spectroscopy as a means to measure age-related quality indexes of Parma ham during processing. Moreover, an explorative application of multiway chemometric methods for autofluorescence landscapes ranging from raw meat to matured hams was used to characterize pure fluorophores in meat samples. In addition, two- and three-way regression methods were applied to autofluorescence data to predict ham age at various processing stages and to search for interdependencies with chemical and sensory properties.

MATERIALS AND METHODS

Sample Material. Lean raw pork from fresh hams was obtained from the local market, whereas Parma hams were from a processing plant in Parma, Italy. Ages of Parma hams ranged from salted (3 months) to matured (11 and 12 months) and further to aged (15 and 18 months). Samples were removed from three leg portions, namely, semimembranosus (SM) and biceps femoris (BF) muscles, plus a slice from the middle section of the leg. Samples for autofluorescence analysis were collected at the processing plant, vacuum packed, and kept in a cold dark room for ~48 h before the time of measurement. They were thermostated in a water bath at 25 °C, and a slice of ~1 cm thickness was cut to fit a sampling cup with a quartz window and an internal diameter of 3.5 cm. A total of 67 meat samples were submitted to duplicate measurement of surface autofluorescence spectroscopy. Of the 67 meat samples, a subset consisting of 34 ham samples from SM and BF muscles, respectively, was used in regression analysis. For this subset of samples sensory and chemical measurements were also included, and the fluorescence data (*X*) together with sensory and chemical data (*Y*) underwent regression analysis.

Measurement of Autofluorescence. Fluorescence spectroscopy was measured using a BioView instrument (Delta Light and Optics, Lyngby, Denmark). The instrument was equipped with a fiber optics measuring probe giving an open-end 180° measuring geometry. The instrument used a pulsed xenon lamp for excitation, and a surface area of ~6 mm in diameter was sampled in each measurement. The sensor was connected to a multichannel fluorescence detector, and 15 excitation and emission filters (each having bandwidths of 20 nm) were employed, measuring excitation from 270 to 550 nm at 20 nm intervals. Accordingly, emission spectra were recorded from 310 to 590 nm at 20 nm intervals. The emission wavelengths were shifted by 40 nm from each excitation wavelength applied.

Chemical/Sensory Analysis. Freshly cut samples from the two muscles were analyzed for instrumental color in terms of CIE L^*, a^*, b^*, hue, and chroma by a Minolta d-508 spectrocolorimeter (Minolta, Osaka, Japan). Visual color assessment of the same samples was performed by an expert panel rating redness intensity on a nine-point scale with extremes (0−9) corresponding to absence and extreme visible redness, respectively. Proximate composition data were measured according to standard methods (*15*), and a proteolysis value was determined on BF muscle as percent ratio between nitrogen soluble in 5% trichloroacetic acid and total nitrogen in the sample (*16*).

Data Analysis. The scope of the data analysis was to explore fluorescence landscapes originating from dried ham for hidden or underlying information and to relate the age of dried ham samples to their fluorescence, chemical, and sensory data. This was fulfilled by fitting a PARAFAC (PARallel FACtor analysis) model to fluorescence data from all types of samples and running bilinear or multiway PLS regression analyses correlating fluorescence data to sample age or chemical/sensory data, respectively.

A three-way array of the fluorescence spectra was used for PARAFAC, which is similar to the bilinear principal component analysis (PCA) or singular value decomposition (SVD), that is, a decomposition method designed for multiway data to reduce dimensionality and extract underlying phenomena (*17*). As in PCA, where a score and a loading vector represent each principal component, each component or factor in PARAFAC consists of one score and two

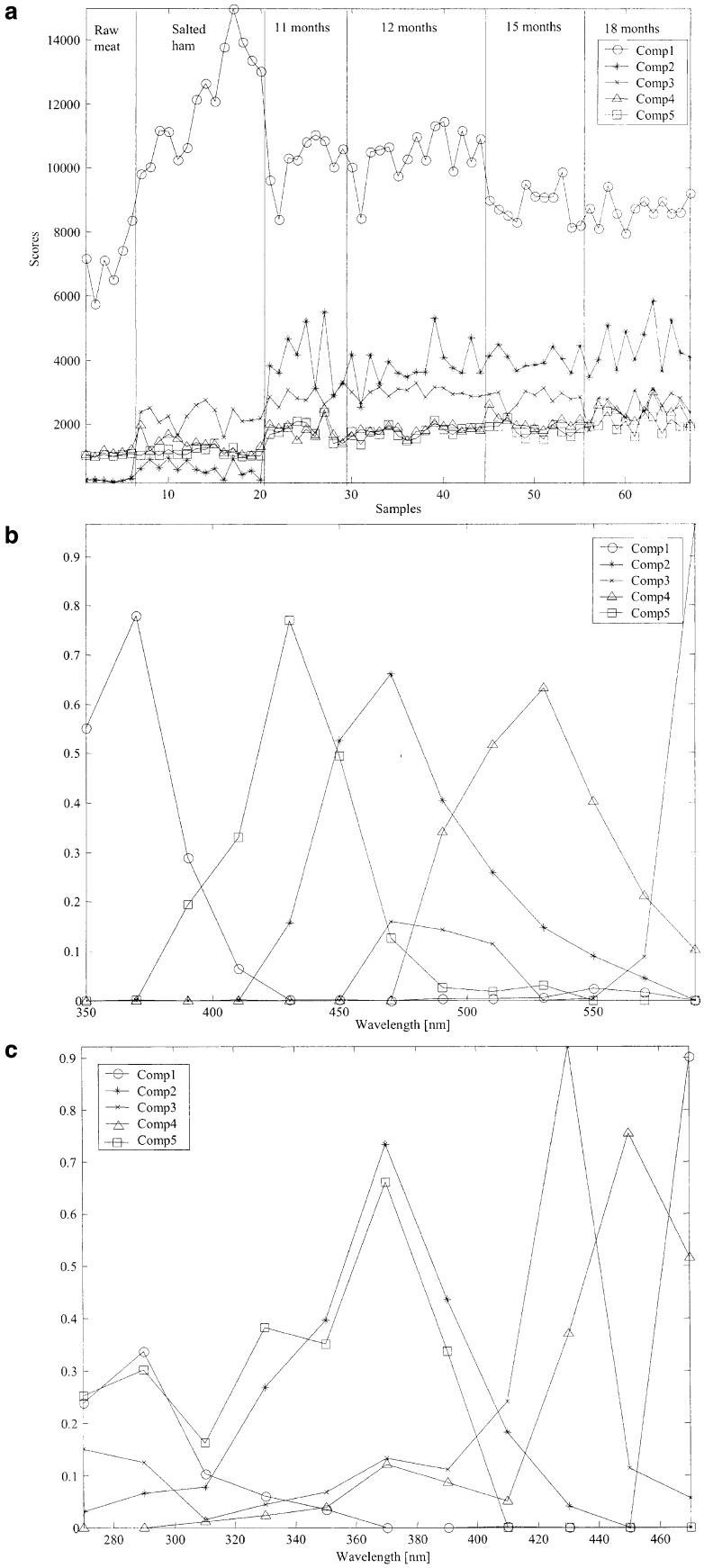

Figure 2. PARAFAC solution with non-negativity in all modes for reduced fluorescence landscapes (13 × 11) of 67 samples ranging from raw meat to Parma ham at different process stages: (a) scores for all samples; (b) loadings in emission mode; (c) loadings in excitation mode corresponding to resolved emission and excitation profiles of fluorophores as estimated by the five-component PARAFAC solution. Scores represent an estimate of the amount of each component in the samples, and the numerical values of scores for every component indicate the portion of variance explained relative to the total variance in all data.

loading vectors (in a three-way data analysis), which represent the three modes of the original data. The PARAFAC modeling was performed using an algorithm in the *N*-way toolbox (*18*) and MATLAB (Mathworks, Natick, MA, version 6.1, release 12.1), in which several criteria can be defined for the PARAFAC model, for example, orthogonality, unimodality, and non-negativity, the latter being most appropriate when fluorescence data are handled. Data were not preprocessed in the three-way analysis of fluorescence data, meaning that neither centering nor scaling was carried out. There were 67 meat samples in the data used for PARAFAC modeling, so the dimensions of the original data array was 67 × 15 × 15 (samples × emission × excitation). The data array was reduced to 67 × 13 × 11 by excluding the two lowest emission wavelengths (<350 nm) and the four highest excitation wavelengths (>470 nm), which contained only a few observations. PARAFAC models with increasing numbers of components were calculated, and the resulting core consistency, which is a measure of data fitting of the model (ideal 100% and generally regarded as acceptable down to 50%), was evaluated (*19*). The number of components was also evaluated by a split-half test in which the 67 samples were divided into two parts, and two PARAFAC models were fitted to each data subset.

To relate sample age to fluorescence data, partial least-squares (PLS) regression analysis was performed on the three-way data array, unfolded matrix, and PARAFAC scores using full cross-validation. In bilinear PLS regression analysis of a matrix *X* containing the independent data and a dependent variable *Y*, factors will be extracted from X to maximize the covariance to the dependent variable, and this principle can also be applied with multiway data arrays, which is named N-PLS. An additional regression analysis was made by adding chemical and sensory parameters to the unfolded two-way fluorescence data, after preliminary tests showed that regression models of unfolded data were better compared to N-PLS regression models using the three-way array of fluorescence data. The unfolded fluorescence data matrix was mean-centered and autoscaled prior to PLS regression, which employed full cross-validation.

RESULTS AND DISCUSSION

Figure 1 shows fluorescence "landscapes" of raw meat, salted, and matured Parma ham samples, and it is seen that major changes in peak wavelengths and intensities are observed for different maturation times. To extract information from and find underlying structures in fluorescence landscapes, a PARAFAC analysis was performed on the three-way data matrix, yielding a five-component model. This model (PARAFAC solution) had a core consistency above 50% and an explained variance of >99%. Validation by a split-half experiment made from two subsets of samples provides the same results (loadings) as with the original 67 samples, and this jack-knife type of analysis then shows that the extracted number of components is correct and that the model is unique.

Results are shown graphically in **Figure 2** as PARAFAC scores and loadings plots, respectively. Scores are an estimate of the "amount" of each component in each sample, and the score plot (**Figure 2a**) supports major differences between several meat classes, with raw meat and salted and maturing hams exhibiting also within-class deviations. The amount of the first component increases from raw to salted meat and then drops to a constant level in 11−12-month-matured hams and further decreases in aged (15−18 months) hams. According to estimated emission and excitation spectra (loading plots, **Figure 2b**,**c**), component 1 is most likely related to aromatic amino acids in proteins having emission and excitation maxima at 370 and 290 nm, respectively. These wavelengths do not match exactly with known values for pure spectra of the fluorescent amino acids tyrosine, tryptophan, or phenylalanine in solution, which may be due to the low spectral resolution of the instrument. Alternatively, the spectral properties of component

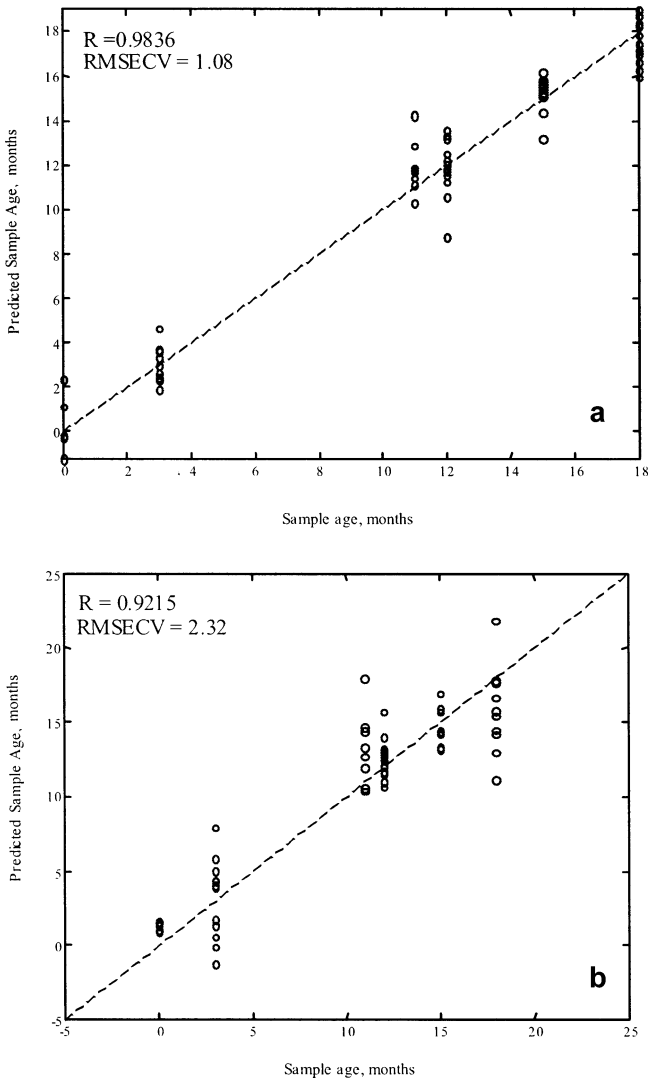

Figure 3. Prediction of sample age or processing stages of meat samples ranging from raw, salted, and maturing hams from PLS regression analysis of actual sample age versus fluorescence data arranged as (a) unfolded spectral data (67 × 101) or (b) scores (67 × 5) obtained from PARAFAC solution (five components, non-negativity constraints in all modes). Results for correlation coefficient and prediction error (root-mean-squared error of cross-validation, RMSECV) were obtained by full cross-validation in the regression analysis including a total of 67 samples.

1 may accordingly be due to a mixture of these amino acids in the meat matrix either bound in protein backbones or as free amino acids. All three aromatic amino acids have been shown to increase in concentration in both BF and SM muscles during 16 months of processing of Iberian dry-cured hams (*20*), and internal quenching phenomena may account for the observed drop in peak intensity when salted meat is compared with matured and aged samples.

The second component, which has excitation and emission maxima at 370 and 470 nm, respectively, increases sharply from salted to matured/aged samples, whereas the third component (430 and >590 nm, respectively) exhibits clear differences between raw and cured meat. It is known that certain inorganic substances, such as chloride, can be detected by reaction with an organic reagent resulting in fluorescence or fluorescence quenching (*21*), and determination of chloride in biological systems has been based on these principles (*22*). The observed emission peak in the red region may accordingly originate from

Table 1. Results from PLS Regression of Chemical/Sensory Parameters and Fluorescence Landscapes from Parma Ham Samples at Various Processing Stages and from Two Muscles, Semimembranosus (SM) and Biceps Femoris (BF)

parameter	samples included (range within sample group)	latent variable no.	% explained variance (*X*/*Y*)	R_{cv}	RMSECV
moisture (%)	all[a] (50.7–68.2)	1	74/36	0.5646	4.04
	SM[a] (50.7–64.0)	2	82/91	0.9185	1.66
	SM, matured[a]	1	21/35	–0.2165	1.91
	BF[a] (59.2–68.2)	2	85/91	0.9141	1.00
	BF, matured[a]	2	79/64	0.6340	0.89
salt (%)	all (3.33–5.90)	1	64/37	0.6735	0.53
	SM (3.41–5.24)	1	66/36	0.6978	0.40
	SM, matured	1		–0.5175	
	BF (3.33–5.90)	1	75/56	0.6707	0.57
	BF, matured	1		–0.1684	
CIE *a**	all (4.48–9.74)	1	74/49	0.7604	0.80
	SM (5.64–9.40)	3	82/27	0.6390	0.77
	SM, matured	1		0.0400	
	BF (4.48–9.74)	6	92/73	0.8645	0.69
	BF, matured	2	69/10	0.3098	0.67
color 1 (arbitrary)	all (3.0–8.5)	1	74/62	0.7373	0.92
	SM (4.0–8.5)	2	74/42	0.5235	1.06
	SM, matured	1		–0.3822	
	BF (3.0–8.5)	2	85/92	0.9356	0.53
	BF, matured	2	78/66	0.6034	0.59
proteolysis value	BF[b] (18.01–39.95)	6	94/87	0.9243	2.07
	BF, matured[b]	6	94/75	0.8428	1.69

[a] Numbers of samples included in each regression analysis: all samples ($n = 34$), all semimembranosus samples ($n = 18$), matured semimembranosus samples ($n = 15$), all biceps femoris samples ($n = 16$), and matured biceps femoris samples ($n = 14$). [b] Numbers of samples included for regression analysis of proteolysis value: all biceps femoris samples ($n = 14$) and matured biceps femoris samples ($n = 12$).

chloride reacting with an organic compound present in the salted and maturing hams.

The fourth and fifth components show little change over the processing span, although the latter seems to discriminate raw and salted from matured/aged meat. The estimated excitation and emission wavelengths for the fifth component may be those of fluorescent tertiary lipid oxidation products, identified both in model systems containing polyunsaturated fatty acid plus glycine (*23*) and in meat (*24*), having excitation and emission maxima at 360 and 440 nm, respectively. Lipolysis and lipid oxidation have been investigated during meat maturation (*25*, *26*), showing that primary (peroxide value) and secondary lipid oxidation products (TBARS) reach a maximum during the process, dropping to negligible levels in the fully matured hams. Several fluorescent lipid peroxidation products from secondary oxidation products, such as aldehydes, and primary amines have been characterized in biological systems (*23*), and most of those were found to be covalently bound to proteins. Therefore, it is likely that tertiary lipid oxidation products emerging late in the maturation of dried hams become detectable in a fluorescence landscape. In a previous study high correlation between TBARS values and fluorescence spectra was found, which enabled the classification between fresh and rancid chicken meat samples (*12*).

Parma hams as well as other dry-cured hams are known to undergo extensive and complex degradation processes yielding free amino acids and small dipeptides (*27*−*30*), which in turn acts as flavor compounds or precursors for volatile flavor compounds (*31*). Lipids are likewise prone to breakdown into low molecular weight compounds (*32*), which are or, after reacting with amines, become fluorophores. Some of these compounds are likely to account for the pronounced changes observed in fluorescence landscapes of samples in this study. Although the present data do not enable resolved components in the PARAFAC model to be assigned to specific chemical compounds, they may be useful in providing a description of the maturation degree of dried hams.

PLS Regression of Spectral Data. To estimate the relationship of fluorescence landscapes of meat samples to their age (months of maturation), PLS regression analyses were performed on the data matrix obtained after the unfolding of the original $67 \times 13 \times 11$ data array and removal of values (columns) above the Rayleigh band. **Figure 3a** shows predicted versus actual age on the basis of original unfolded data (67×101), with a root-mean-squared error of cross-validation (RMSECV) of 1.08 months and $R = 0.98$. Results were less satisfactory (RMSECV $= 1.34$ and $R = 0.97$) when regression analysis was made of the three-way data array of fluorescence landscapes ($67 \times 13 \times 11$). This observation indicates that the relatively high number of missing values (~30%) in the fluorescence landscapes arranged as a three-way array impairs the final result of this particular model, whereas the unfolded matrix containing no missing values provides a better description of the original data. Also, scores from the previously presented five-component constrained PARAFAC solution were correlated to sample age by the same statistical procedure (**Figure 3b**), leading to a prediction error of 2.3 months, or twice that found with two- or three-way PLS regression of the original spectral data.

Additional PLS regression analyses performed either with unfolded fluorescence data (two-way) or an array of fluorescence landscapes (three-way) were focused only on matured samples, to estimate if a fluorescence-based model can help discriminate between hams at the last processing stages (11−18 months). Results obtained from two- and three-way PLS regression models (both having RMSECV $= 1.1$ months and $R = 0.92$) demonstrate that the subset of fluorescence data containing only maturing/aging samples conforms better to trilinearity compared to the complete data set (67 samples), where differences in correlation coefficient and RMSECV for the two- and three-way PLS regression model are observed. When scores from the

PARAFAC model were used, a poor correlation was obtained ($R = 0.76$), with an error (RMSECV) of 1.8 months.

PLS Regression of Spectral, Chemical, Physical, and Sensory Data. To search for relationships between spectral (fluorescence), chemical, physical, and sensory data, the fluorescence data were used as *X* data and chemical (moisture and salt), physical (CIE *a**), and sensory (visual color) measurements from the same ham samples were used as *Y* data, resulting in a data set of 34 samples ranging from salted to aged Parma ham (18 month). Results of PLS regression analyses, performed independently for SM and BF muscles (**Table 1**), show that fluorescence from BF samples was better correlated to most of the chemical/sensory parameters, for example, salt, instrumental redness (CIE *a**), and visual color. A previous correlation study between sensory and chemical parameters of Parma ham found moisture to be inversely related to taste and firmness, whereas instrumental color exhibited poor correlation to sensory color (*7*), which could be due to noninformative instrumental methods applied. The present fluorescence landscapes seem to contain information relating to both instrumental (CIE *a**) and sensory color measures, as shown by good correlation coefficients of CIE *a** versus fluorescence data for both muscles, whereas sensory color is especially well correlated in BF samples.

It can also be observed that fluorescence landscapes from SM samples are similarly well correlated to moisture, as are BF muscles, but RMSECV for prediction of moisture in BF is 33% lower than in SM samples. Because the relative error for moisture analysis in dried ham is ~0.4% and because the intervals for moisture in SM and BF are 68−59 and 64−51%, respectively, the estimated prediction error, ranging from 1.91 to 0.89% when separate muscles are analyzed, seems to be satisfactory. The results obtained with the two types of muscles may in part differ because SM and BF are located at the exterior and interior of the whole ham, respectively. This means that SM during processing will develop lower moisture and salt contents as moisture escapes from the surface and salt diffuses toward the interior, where BF is located, and as a consequence the proteolysis will in general be most pronounced in BF. Compared with previous PLS regression studies of chemical-to-sensory or chemical-to-instrumental relationships in dried hams (7), relatively large proportions of variances could be explained (on average $X > 70\%$ and $Y > 50\%$) in this investigation, indicating that fluorescence data contain relevant information concerning the chemical composition of dry-cured hams.

In conclusion, the present study shows that the PARAFAC model or PLS regression based on fluorescence landscapes allows discrimination between raw meat, salted meat, and matured/aged ham, plus prediction of chemical, physical, or sensory parameters in the dried ham. Previous studies using fluorescence data to evaluate meat toughness (*8*), quantify connective tissue (*9*), or estimate oxidation (*12*) in meat have monitored fluorescence at only three to four excitation wavelengths, and these applications may also benefit from the use of fluorescence landscapes in combination with multiway chemometric techniques.

Future work with the prediction of chemical/sensory parameters by fluorescence spectroscopy will focus on the ability to identify defective hams early in the process. Furthermore, dry-cured hams from other processing plants will be included to validate the results obtained in the present study.

ACKNOWLEDGMENT

This study was partly sponsored by an EU innovation program (OPUS, IN30905I) which aims at adapting and applying advanced fluorescence measurement techniques for on-line analysis in traditional food industries. Dr. Rasmus Bro is gratefully acknowledged for valuable advice during the data analysis.

LITERATURE CITED

(1) Arnau, J.; Guerrero, L.; Casademont, G.; Gou, P. Physical and chemical changes in different zones of normal and PSE dry-cured ham during processing. *Food Chem.* **1995**, *52*, 63−69.

(2) Gil, M.; Guerrero, L.; Sarraga, C. The effect of meat quality, salt and aging time on biochemical parameters of dry-cured Longissimus dorsi muscle. *Meat Sci.* **1999**, *51*, 329−337.

(3) Virgili, R.; Schivazappa, C.; Parolari, G.; Bordini, C. S.; Degni, M. Proteases in fresh pork muscle and their influence on bitter taste formation in dry-cured ham. *J. Food Biochem.* **1998**, *22*, 53−63.

(4) Sentandreu, M. A.; Toldra, F. Dipeptidyl peptidase activities along the processing of Serrano dry-cured ham. *Eur. Food Res. Technol.* **2001**, *213*, 83−87.

(5) Parolari, G. Achievements, needs and perspectives in dry-cured ham technology: The example of Parma ham. *Food Sci. Technol. Int.* **1996**, *2*, 69−78.

(6) Schivazappa, C.; Degni, M.; Costa, L. N.; Russo, V.; Buttazzoni, L.; Virgili, R. Analysis of raw meat to predict proteolysis in Parma ham. *Meat Sci.* **2002**, *60*, 77−83.

(7) Virgili, R.; Parolari, G. Quality control in the meat industry by multivariate statistics. The case of raw ham. *Meat Sci.* **1991**, *29*, 83−96.

(8) Swatland, H. J.; Findlay, C. J. On-line probe prediction of beef toughness, correlating sensory evaluation with fluorescence detection of connective tissue and dynamic analysis of overall toughness. *Food Qual. Pref.* **1997**, *8*, 233−239.

(9) Wold, J. P.; Lundby, F.; Egelandsdal, B. Quantification of connective tissue (hydroxyproline) in ground beef by autofluorescence spectroscopy. *J. Food Sci.* **1999**, *64*, 1−7.

(10) Swatland, H. J. Connective and adipose tissue detection by simultaneous fluorescence and reflectance measurements with an on-line meat probe. *Food Res. Int.* **2000**, *33*, 749−757.

(11) Egelandsdal, B.; Wold, J. P.; Sponnich, A.; Neegard, S.; Hildrum, K. I. On attempts to measure the tenderness of Longissimus Dorsi muscles using fluorescence emission spectra. *Meat Sci.* **2002**, *60*, 187−202.

(12) Wold, J. P.; Mielnik, M. Nondestructive assessment of lipid oxidation in minced poultry meat by autofluorescence spectroscopy. *J. Food Sci.* **2000**, *65*, 87−95.

(13) Næs, T.; Baardseth, P.; Helgesen, H.; Isaksson, T. Multivariate techniques in the analysis of meat quality. *Meat Sci.* **1996**, *43*, S135−S149.

(14) Munck, L.; Nørgaard, L.; Engelsen, S. B.; Bro, R.; Andersson, C. A. Chemometrics in food science—a demonstration of the feasibility of a highly exploratory, inductive evaluation strategy of fundamental scientific significance. *Chemom. Intell. Lab. Syst.* **1998**, *44*, 31−60.

(15) AOAC. *Official Methods of Analysis of AOAC International*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990.

(16) Careri, M.; Mangia, A.; Barbieri, G.; Bolzoni, L.; Virgili, R.; Parolari, G. Sensory property relationships to chemical-data of Italian-type dry-cured ham. *J. Food Sci.* **1993**, *58*, 968−972.

(17) Bro, R. PARAFAC. Tutorial and applications. *Chemom. Intell. Lab. Syst.* **1997**, *38*, 149−171.

(18) Andersson, C. A.; Bro, R. The N-way Toolbox for MATLAB. *Chemom. Intell. Lab. Syst.* **2000**, *52*, 1−4.

(19) Bro, R.; Kiers, H. A. L. A new efficient method for determining the number of components in PARAFAC models. *J. Chemom.* **2002**, submitted for publication.

(20) Cordoba, J. J.; Rojas, T. A.; Gonzalez, C. G.; Barroso, J. V.; Bote, C. L.; Asensio, M. A. Evolution of free amino acids and amines during ripening of Iberian cured ham. *J. Agric. Food Chem.* **1994**, *42*, 2296−2301.
(21) Fernandez-Gutierrez, A.; de la Peña, A. M. Determinations of inorganic substances by luminescence methods. In *Molecular Luminescence Spectroscopy. Methods and Applications: Part I*; Schulman, S. G., Ed.; Wiley: New York, 1985; pp 371−546.
(22) Sonawane, N. D.; Thiagarajah, J. R.; Verkman, A. S. Chloride concentration in endosomes measured using a ratioable fluorescent Cl^- indicator. Evidence for chloride accumulation during acidification. *J. Biol. Chem.* **2002**, *277*, 5506−5513.
(23) Shimasaki, H. Assay of fluorescent lipid peroxidation products. *Methods Enzymol.* **1994**, *233*, 338−346.
(24) Stapelfeldt, H.; Bjørn, H.; Skovgaard, I. M.; Skibsted, L. H.; Bertelsen, G. Warmed-over flavor in cooked sliced beef—Chemical analysis in relation to sensory evaluation. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 203−208.
(25) Vestergaard, C. S.; Schivazappa, C.; Virgili, R. Lipolysis in dry-cured ham maturation. *Meat Sci.* **2000**, *55*, 1−5.
(26) Vestergaard, C. S.; Parolari, G. Lipid and cholesterol oxidation products in dry-cured ham. *Meat Sci.* **1999**, *52*, 397−401.
(27) Flores, J. Mediterranean vs northern European meat products. Processing technologies and main differences. *Food Chem.* **1997**, *59*, 505−510.
(28) Toldra, F. Proteolysis and lipolysis in flavour development of dry-cured meat products. *Meat Sci.* **1998**, *49*, S101−S110.
(29) Virgili, R.; Parolari, G.; Bordini, C. S.; Schivazappa, C. Free amino acids and dipeptides in dry-cured ham. *J. Muscle Foods* **1999**, *10*, 119−130.
(30) Hansen-Møller, J.; Hinrichsen, L.; Jacobsen, T. Evaluation of peptides generated in Italian-style dry-cured ham during processing. *J. Agric. Food Chem.* **1997**, *45*, 3123−3128.
(31) Hinrichsen, L. L.; Pedersen, S. B. Relationship among flavor, volatile compounds, chemical changes, and microflora in Italian-type dry-cured ham during processing. *J. Agric. Food Chem.* **1994**, *43*, 2932−2940.
(32) Chizzolini, R.; Novelli, E.; Zanardi, E. Oxidation in traditional Mediterranean meat products. *Meat Sci.* **1998**, *49*, S87−S99.

Received for review May 10, 2002. Revised manuscript received December 2, 2002. Accepted December 16, 2002.

JF025662H