

Monitoring the Time and Temperature History of Frozen Hake (*Merluccius merluccius*, L.) Muscle by FTIR Spectroscopy of the Lipid Fraction

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Abstract The objective was to model the time and temperature changes of the lipid fraction of hake muscle by mid-infrared spectroscopy. Fillets of a total of 90 hake (*Merluccius merluccius*, L.), stored at -10 , -20 , -30 or -80 °C for up to 150 weeks, were used and lipids analysed by attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (FTIR). Three spectral regions corresponding to $\nu(\text{C-H})$ vibrations ($3,100\text{--}2,750\text{ cm}^{-1}$), $\nu(\text{C=O})$ vibrations from ester and carboxylic groups ($1,800\text{--}1,670\text{ cm}^{-1}$) and $\nu_{as}(\text{PO}_2^-)$ phospholipid vibrations ($1,330\text{--}1,127\text{ cm}^{-1}$) were studied separately. Principal component scores were used as the response variables for the kinetic modelling. Three models were obtained, each per region, and the best one resulted from the region corresponding to the phosphorous moiety of the phospholipids with $R^2=0.93$. They kept a close relationship with other reported quality parameter such as Kramer shear resistance which suggests their potential for the estimation of frozen storage time and temperature in fishery products.

Keywords Fish quality · Hake · Frozen storage · FTIR · Kinetic modelling · Arrhenius

Introduction

The fishery sector has been demanding fast instrumental methods to estimate the quality and shelf life of frozen fish products (Jørgensen et al. 2003). It is well known that factors

such as species, season, type of catch or slaughtering, *post mortem* handling, freezing and frozen storage conditions and the interactions of all these factors may affect the fish flesh structure, composition and functionality, and as a consequence, their quality and shelf life.

Frozen fish muscle deterioration involves a series of alterations in protein secondary and tertiary structure, water distribution and mobility, lipid oxidation and lipolysis. In lean fish species such as hake, ultrastructural changes have been observed in fibre organization due to the pressure exerted by ice crystals. Intermyofibrillar spaces may become reduced, the sarcoplasmic reticulum gradually compressed into a thin layer and a reduction in the inter-filament distances can take place (Herrero et al. 2005). These changes lead to loss of water holding capacity and increase in shear resistance in hake muscle (Sánchez-Valencia et al. 2014).

Mid-infrared spectroscopy has been used to investigate the lipid deterioration process in hake muscle frozen stored in conditions where a high range of texture change was achieved. Among the structural and compositional features observed by Fourier transform infrared spectroscopy (FTIR), the changes in the carbonyl stretching bands from esters and carboxylic acids and the disappearance of the bands from the asymmetric stretching PO_2^- group showed strong time dependence at frozen storage in hake (Sánchez-Alonso et al. 2012a). This suggested phospholipid hydrolysis as contributor to the toughening of hake upon storage (Herrero et al. 2005; Sánchez-Alonso et al. 2012a). These changes in the lipid fraction suggest that FTIR spectroscopy may provide markers for modelling the time and temperature history of frozen fillets.

Several methods have been proposed to predict the frozen storage time of hake products at a fixed temperature, including the Quality Index Method (QIM) (Herrero et al. 2003) based on sensory inspection, stress relaxation test (Herrero and Careche 2005), visible spectroscopy (Heia et al. 2003), a

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combination of methods such as apparent viscosity of muscle homogenates, puncture test, and Kramer shear resistance (Herrero and Careche 2006), and a LF-NMR-based method (Sánchez-Alonso et al. 2012b). The spectroscopic techniques are preferred for fish quality and safety evaluation, and the analysis of data is usually performed by chemometrics (Chen et al. 2013).

The temperature dependence of the rate of the physical changes or chemical reactions can be modelled by several ways. One of them is the Arrhenius equation which has been applied for physical and chemical phenomena (Taoukis et al. 1997), and in many food systems, it has been used to estimate shelf life (e.g. Labuza 1984; Fu and Labuza 1993; Taoukis et al. 1997). Few reports in the literature use this approach for frozen seafood products (Tsironi et al. 2009; Sánchez-Valencia et al. 2014) as opposed to their extended use in chilled storage (i.e. Zhang et al. 2011) or during thermal processing (i.e. Kong et al. 2007).

Principal component analysis (PCA) is a commonly used data compression and visualization tool, reducing a data set into a small number of new, noncorrelated variables on each of which a score for each sample is calculated (Afifi and Clark 1984) and graphical display of these scores can often reveal patterns within a data set. The aim of this work was to find spectroscopic markers for the estimation of quality and shelf life of frozen hake muscle (*Merluccius merluccius* L.) as affected by temperature and time. For that, we chose FTIR spectroscopy of the lipid fraction, divided the spectra into three regions known to carry specific physical chemical information on the changes in fish lipids, performed principal component analysis on these spectral regions and used the PC scores as the parameters of study for kinetic modelling.

Material and Methods

Fish Samples

Hake (*M. merluccius*) were captured in the Northeast Atlantic (FAO area 27, division VIa) in November 2009. They were gutted and iced on board and landed on Puerto de Celeiro (Galicia, Spain) 24 h after capture. Immediately after landing, they were weighted (average 1.81 ± 0.19 kg), visually inspected and the scales were removed. The fish in post-rigour condition were beheaded, washed with iced water to remove blood, rests of viscera and so on and allowed to drain in stainless steel racks. Then, individuals were filleted manually (skin left on) and visually inspected to make sure of the complete removal of any rests of bones, scales or viscera and individually packed in Ariex-3 plastic bags (polyamide20/polyethylene70, thickness 90 μ , Industrias Plásticas Arias S.L., Lugo, Spain), labelled and sealed with no vacuum in a packaging and sealing equipment (model VP-800, Ramón,

Vilassar de Dalt, Barcelona, Spain). The temperature during handling was always kept under 7 °C. Fillets were frozen in a blast freezer (RF 201 AB, Fagor, Mondragón, Spain) for 1 h until the fish reached a core temperature of -20 °C, placed in polystyrene boxes with dry ice and immediately transported from Puerto de Celeiro to the ICTAN laboratory in Madrid. They were stored at -10 , -20 , -30 and -80 °C. Those stored at -10 °C were sampled up to 16 weeks, and the ones at -20 and -30 °C, for up to 104 and 147 weeks, respectively. Fillets from 20 frozen fish were kept at -80 °C and analysed after 1, 24, 106 and 150 weeks of storage. For each storage period, fillets from three individuals were thawed in a cold room at 2 °C and subjected to analyses as described below.

Lipid Extraction

Lipids were extracted from the dorsal part of hake fillets according to the method of Bligh and Dyer (1959) in duplicate (duplicate extractions from each specimen, making up a total of six oil extractions for each storage period). Lipid content was determined gravimetrically and expressed on a wet weight basis (g lipid/100 g muscle). Sample (40 g) was homogenized with 120 ml of a methanol/dichloromethane mixture (2:1) at the speed of 13,500 rpm for 2 min using a homogenizer (T 25 digital Ultra-Turrax, IKA-Werke GmbH & Co. KG, Germany). The homogenate was treated with 40 ml of dichloromethane, homogenized for 1 min and decanted into test tubes through a Whatman No. 1 filter paper. Then, 40 ml of distilled water was added and the mixture was shaken manually and maintained at 0 °C overnight. The dichloromethane phase was transferred into separation flasks, and after sodium chloride addition (0.5 %, 1:2 v/v), it was shaken vigorously. The organic phase was drained off into Erlenmeyer flasks and about 2–4 g of sodium sulphate anhydrous was added, shaken and filtered through a Whatman No. 1 filter paper into a previously dried round-bottom flask. The solvent was evaporated at 30–40 °C using a Büchi rotary R-evaporator (Büchi Labortechnik AG, Switzerland) up to a volume of 10 ml that was stored in aliquots of 2 ml at -80 °C until analyses.

Fourier Transform Infrared Spectroscopy Analysis

All spectra were acquired using a Perkin-Elmer model Spectrum-400 FT-MIR spectrometer (PerkinElmer España, S.L., Madrid, Spain) with a horizontal attenuated total reflectance (ATR) plate crystal of ZnSe and diamond equipped with a detector of deuterated triglycine sulphate (DTGS) and with KBr window. FTIR spectra were collected in mid-IR region of $4,000$ – 650 cm^{-1} by accumulating 16 scans, at 0.5 cm^{-1} scan speed and 2 cm^{-1} resolution with strong apodization. The measurements were directly carried out by uniformly spread oil samples throughout the ATR surface at controlled room

temperature. The spectrum of each sample was ratioed against a background obtained with a dry and empty ATR crystal. Before acquiring each spectrum, the surface of ATR plate was carefully cleaned by twice wiping with heptane and drying with soft tissue and a new reference air background spectrum was taken. At least five spectra for each oil sample were collected. PE Spectrum software package (Version 6.3.2.0151, PerkinElmer) was used for instrument control and data acquisition.

Data Analysis

FTIR data processing was done by the GRAMS/AI software from Thermo Electron Corporation (Waltham, MA, USA). The repeated FTIR measurements per fish were averaged and taken as the replicate for displaying the evolution over time and temperature. The number of individuals per each storage time and temperature (replicates) was $N=3$.

Three spectral regions corresponding to $\nu(\text{C-H})$ vibrations ($3,100\text{--}2,750\text{ cm}^{-1}$), $\nu(\text{C=O})$ vibrations from ester and carboxylic groups ($1,800\text{--}1,670\text{ cm}^{-1}$) and $\nu_{as}(\text{PO}_2^-)$ phospholipid vibrations ($1,330\text{--}1,127\text{ cm}^{-1}$) were studied separately. Factor analysis was performed in each of them, and the principal component (PC) scores were taken as the response variables for the analysis described below.

For a first estimation of the kinetic reaction parameters for each temperature, the data obtained per storage time were averaged and fitted to zero or to first-order kinetics. For a zero-order reaction, the equation can be expressed as

$$A_0 - A = kt \quad (1)$$

and for a first-order reaction, as

$$A_0/A = \exp(kt) \quad (2)$$

where A_0 is the value of the response variable (PC1 or PC2) at time 0; A the value of this response variable at time t and k is the rate constant.

The temperature dependence of the response variables can be analysed with an Arrhenius type equation (van Boekel 2008):

$$k = k_A \exp(-B/T) \quad (3)$$

where k is the rate constant; T the absolute temperature in Kelvin; k_A the pre-exponential factor and B is a constant. Time and temperature can be modelled in one-step by using nonlinear regression instead of using a two-step approach in which the kinetic parameters for each temperature are calculated first and then applied to estimate the temperature dependence. The one-step approach increases the degrees of freedom and results in a more accurate estimation of k at each

temperature, giving narrower confidence intervals (Taoukis et al. 1997; van Boekel 2008).

Secondly, in agreement with previous works (Taoukis et al. 1997; van Boekel 2008), the standard error of k_A can be lowered by applying the model to the relevant temperature range (although the model itself may be applicable to a wider range). The reference rate constant (k_{ref}) usually corresponding to an average of the temperature range characteristic of the process has in this way a more relevant meaning, and the applicability of the equation is maximal within the range of temperatures of interest. The nonlinear regression by using the T_{ref} approach is written as follows:

For zero-order kinetics:

$$A = A_0 + k_{\text{ref}}t \exp[-B(1/T - 1/T_{\text{ref}})] \quad (4)$$

For first-order kinetics:

$$A = A_0 \exp[-k_{\text{ref}}t \exp(-B(1/T - 1/T_{\text{ref}}))] \quad (5)$$

where T_{ref} is the reference temperature and k_{ref} is the rate constant at this temperature. In this case, the chosen T_{ref} was 253 K ($-20\text{ }^\circ\text{C}$), close to the maximum recommended storage temperature of $-18\text{ }^\circ\text{C}$. PCs were transformed in order to perform first-order kinetics by performing a scale change from the original $(-a, a)$ to $(-a-1, a+a+1)$. Starting values for the nonlinear regression were calculated using the parameters calculated by applying Eq. 3 and the rate constants obtained from Eqs. 1 or 2. A_0 , k_{ref} and B parameters were estimated with data from all storage times and temperatures ($N=90$ fish) using the Nonlinear Regression programme (Levenberg-Marquardt algorithm) with the SPSS software (IBM SPSS statistics version 20, IBM Corporation, 2011).

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Results

Proximate analyses of hake muscle from this experiment have been published elsewhere (Sánchez-Valencia et al. 2014) and were within the values previously reported for this species (Careche and Tejada 1990; Sánchez-Alonso et al. 2012b).

Fig. 1 Infrared spectra ($3,500\text{--}650\text{ cm}^{-1}$) of lipids extracted from hake muscle stored at **a** $-10\text{ }^{\circ}\text{C}$ for 1 (upper line), 5 (medium line) and 16 (lower line) weeks, **b** $-20\text{ }^{\circ}\text{C}$ for 2 (upper line), 25 (medium line) and 104 (lower line) weeks and **c** $-30\text{ }^{\circ}\text{C}$ for 19 (upper line) and 105 (lower line) weeks

Influence of Storage Time and Temperature on Selected FTIR Bands

Figure 1 shows representative FTIR spectra of lipids extracted from hake fillets at different storage temperatures and times. The $3,000\text{--}2,800\text{ cm}^{-1}$ region is characteristic of the symmetric and asymmetric CH stretching vibrations of the methylene and methyl groups in the fatty acid chains. At the $1,800\text{--}1,670\text{ cm}^{-1}$ region, the carbonyl stretching vibrations from esters and carboxylic acids appear. The $1,500\text{--}900\text{ cm}^{-1}$ frequency interval is also known as the fingerprint region which includes a band near $1,232\text{ cm}^{-1}$ resulting from phosphate motion in phospholipids. The structural changes in the infrared spectra as a function of time and storage temperature are described elsewhere (Sánchez-Alonso et al. 2013).

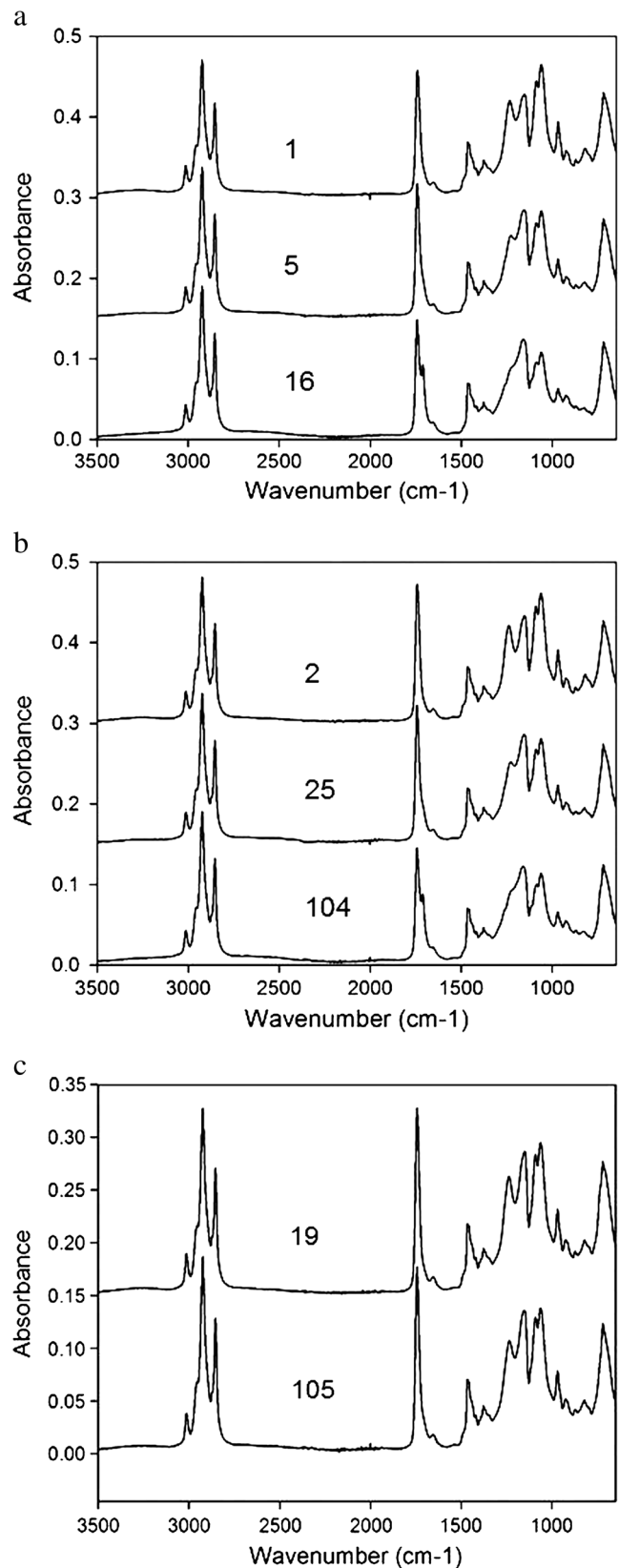
Particular wavenumbers representative of functional groups susceptible of modification with oxidative or lipolytic changes were selected for a preview of their evolution with temperature and time (Fig. 2). The changes in the infrared spectral bands in the upper region are used for monitoring the development of lipid oxidation. No clear trends in the ratios between these bands were observed (Fig. 2a) which is consistent with previous results (Sánchez-Alonso et al. 2012a). Strong time and temperature dependence of the $1,711$ to $1,742\text{ cm}^{-1}$ ratio could be observed (Fig. 2b). When lipolysis occurs, a band generated by the carboxyl groups in the free fatty acids is released, and appears near $1,712\text{ cm}^{-1}$, together with a gradual decrease in the absorption intensity of the band near $1,742\text{ cm}^{-1}$ (Sánchez-Alonso et al. 2012a). Time and temperature dependence of a band at $1,231\text{ cm}^{-1}$ (Fig. 3c), assigned to asymmetric stretching motion of phosphate group in phospholipids, have been explained in terms of phospholipid hydrolysis (Sánchez-Alonso et al. 2013).

Factor Analysis

Three spectral regions in the IR were used for factor analysis: $3,100\text{--}2,750$, $1,800\text{--}1,670$ and $1,330\text{--}1,127\text{ cm}^{-1}$. Factor analysis showed that in all three ranges, 3 PC were sufficient to explain at least 95 % of the total variance (Table 1). These PCs showed strong time-temperature dependence (Fig. 3), which was higher than that of individual parameters shown in Fig. 2. This was especially evident for the $3,100\text{--}2,750\text{ cm}^{-1}$ region.

Kinetic Models of PC Scores

The results presented above indicate a clear relationship between PC scores and storage time and temperature. Table 2



shows the parameters of the equations resulting from adjusting to zero or to first-order kinetics (Eqs. 1 and 2) using averaged

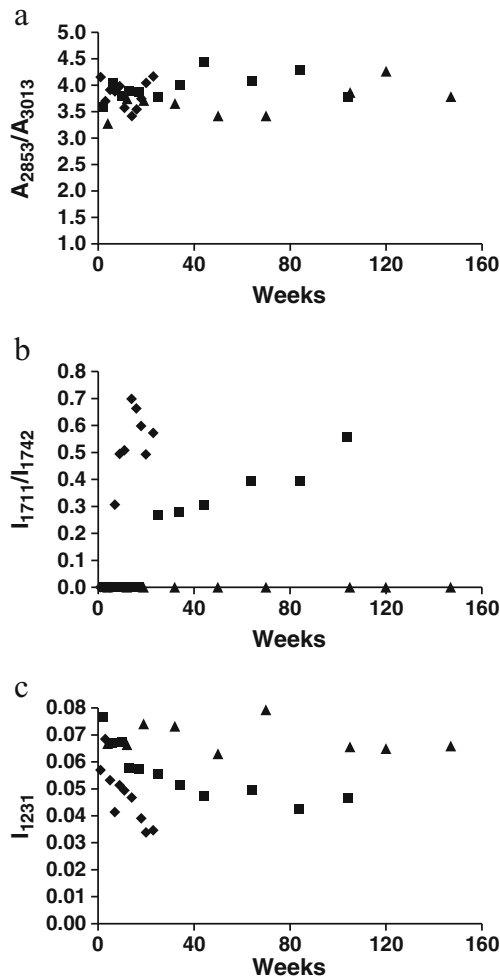


Fig. 2 FTIR spectral features of lipids extracted from hake fillets during frozen storage at $-10\text{ }^{\circ}\text{C}$ (diamond), $-20\text{ }^{\circ}\text{C}$ (square) and $-30\text{ }^{\circ}\text{C}$ (triangle). **a** Area ratio of the $\nu_s(\text{CH}_2)$ and cis-olefinic $\nu(\text{C-H})$ bands ($2,853/3,013\text{ cm}^{-1}$), **b** intensity ratio of the $1,711/1,742\text{ cm}^{-1}$ $\nu\text{-C=O}$ bands of carboxylic acids and ester groups, respectively, and **c** intensity value of the $1,231\text{ cm}^{-1}$ $\nu_{\text{as}}(\text{PO}_2^-)$ of phospholipids. Average values per storage time and temperature

data per storage time and temperature. The coefficients of determination were in general good for data at -10 and $-20\text{ }^{\circ}\text{C}$ whereas R^2 s were poorer at the lowest temperatures which is to be expected due to the low reaction rate and the given standard errors. The rate constants were used for estimating the values of the pre-exponential factor k_{ref} and the constant B , according to the modified Arrhenius Eqs. 4 and 5, and shown in Table 3. No significant differences could be found for the temperature dependence according to the B values and their standard deviations. Therefore, we assumed that the temperature dependency was not different in all three regions, but the function that better represented the variability of the data was the one obtained in the $1,330\text{--}1,127\text{ cm}^{-1}$ region. For this function, there was good agreement between predicted and observed values (Fig. 4) suggesting its potential suitability for quality management and estimation of remaining shelf life.

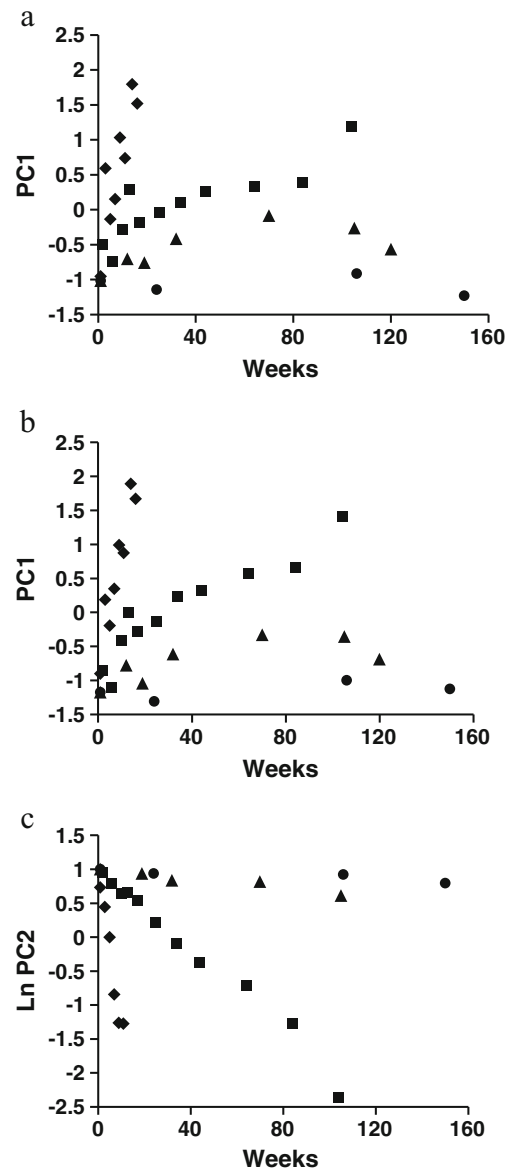


Fig. 3 Changes of PC scores of the regions corresponding to **a** $\nu(\text{C-H})$ groups ($3,100\text{--}2,750\text{ cm}^{-1}$), **b** ester and carboxylic $\nu(\text{C=O})$ groups ($1,800\text{--}1,670\text{ cm}^{-1}$) and **c** $\nu_{\text{as}}(\text{PO}_2^-)$ of the phospholipids ($1,330\text{--}1,127\text{ cm}^{-1}$) of hake fillets stored at $-10\text{ }^{\circ}\text{C}$ (diamond), $-20\text{ }^{\circ}\text{C}$ (square), $-30\text{ }^{\circ}\text{C}$ (triangle) and $-80\text{ }^{\circ}\text{C}$ (circle)

Discussion

There is a wide agreement about the potential of spectroscopic techniques for fish quality and safety evaluation (e.g. Carmona et al. 2010; Uddin and Okazaki 2010; Cheng et al. 2013) since they can ideally be nondestructive, fast and cost-effective. Moreover, in a spectrum, information of several chemical procedures is being provided, and mid-infrared spectroscopy has the advantage that peaks in a spectrum are exclusive for a particular type of organic bond (Cheng et al. 2013).

Table 1 Factor analysis: percentage of explained variance for the spectral regions corresponding to $\nu(\text{C-H})$ vibrations (3,100–2,750 cm^{-1}), ester and carboxylic $\nu(\text{C=O})$ vibrations (1,800–1,670 cm^{-1}) and $\nu_{\text{as}}(\text{PO}_2^-)$ phospholipid vibration (1,330–1,127 cm^{-1})

Component	Accumulated explained variance (%)		
	3,100–2,750 cm^{-1}	1,800–1,670 cm^{-1}	1,330–1,127 cm^{-1}
1	64	48	73
2	88	77	96
3	95	95	98
4	99	99	99
5		100	99
6			100

Free fatty acids formed by hydrolysis of triacylglycerols and phospholipids and lipid oxidation products have been reported to have an effect on the techno-functional and textural characteristics of fish muscle during frozen storage (Shenouda 1980; de Koning and Mol 1991; Haard 1992; Aubourg et al. 1999, 2007; Roldan et al. 2005; Kolakowska 2010). The detrimental flavour, texture and colour changes due to lipid oxidation products are very well documented (e.g. Undeland 1997), and there are also evidences on the changes in taste due to free polyunsaturated fatty acids (Refsgaard et al. 2000).

It is well known that two of the most important processing factors affecting the quality of frozen fish products are the storage time and temperature. Whereas spectroscopic methods have been developed to monitor the frozen storage time at a fixed temperature (e.g. Heia et al. 2003; Herrero

Table 3 Regression parameters (\pm standard error) for PC1 scores of the spectral region corresponding to the $\nu(\text{C-H})$ vibrations (3,100–2,750 cm^{-1}), PC1 scores of the ester and carboxylic $\nu(\text{C=O})$ vibrations (1,800–1,670 cm^{-1}) and PC2 scores of the $\nu_{\text{as}}(\text{PO}_2^-)$ phospholipid vibrations (1,330–1,127 cm^{-1}) of hake fillets stored at $-10, -20, -30$ and -80°C

	Zero order PC1 3,100–2,750 cm^{-1}	Zero order PC1 1,800–1,670 cm^{-1}	First order PC2 1,330–1,127 cm^{-1}
A_0	-0.698 ± 0.069	-0.752 ± 0.059	2.738 ± 0.035
k_{ref}	0.018 ± 0.002	0.021 ± 0.002	0.022 ± 0.001
B	$14,257 \pm 760$	$12,032 \pm 549$	$13,584 \pm 350$
R^2	0.57	0.70	0.93

These regression parameters were extracted from the equations of zero ($A = A_0 + k_{\text{ref}} t \exp[-B(1/T - 1/T_{\text{ref}})]$) and first order ($A = A_0 \exp[-k_{\text{ref}} t \exp(-B(1/T - 1/T_{\text{ref}}))]$) with a reference temperature (T_{ref}) set at 253 K. A is the response variable (PC1 or PC2), k_{ref} is the rate constant (week^{-1}) at T_{ref} , B is a constant (K), t is the storage time (weeks) and T the absolute temperature (K). A_0 is the initial value of the response variable. $N=90$ fish

et al. 2004; Sánchez-Alonso et al. 2012b), the study of combining the effect of these two factors has been much limited and by means of chemometrics (Kent et al. 2005). Kinetic modelling of spectroscopic data has the advantage of providing energies of activation or constants of chemical reactions that can be related with previous reported data. Therefore, we aimed at developing kinetic models in order to see the potential of the combination of both the spectroscopic method and data analysis approach, to address the estimation of quality and remaining shelf life.

Principal component analysis allowed us to reduce a large number of variables to a smaller number containing almost the same amount of information. Some of these PC

Table 2 Parameters (\pm standard error) of the regression equations of zero ($A_0 - A = kt$) and first order ($A_0/A = \exp(kt)$) for PC1 scores of the spectral region corresponding to the $\nu(\text{C-H})$ vibrations (3,100–2,750 cm^{-1}), PC1

scores of the ester and carboxylic $\nu(\text{C=O})$ vibrations (1,800–1,670 cm^{-1}) and PC2 scores of the $\nu_{\text{as}}(\text{PO}_2^-)$ phospholipid vibration (1,330–1,127 cm^{-1}) of hake fillets stored at $-10, -20, -30$ and -80°C

Kinetic model		Temperature $^\circ\text{C}$			
		$-10 (N=8)$	$-20 (N=11)$	$-30 (N=7)$	$-80 (N=4)$
Zero order	A_0	0.643 ± 0.321	-0.418 ± 1.20	-0.760 ± 0.157	-1.039 ± 0.126
PC1	k	0.150 ± 0.033	0.013 ± 0.002	0.004 ± 0.002	-0.001 ± 0.001
3,100–2,750 cm^{-1}	R^2	0.769	0.768	0.397	0.069
Zero order	A_0	-0.785 ± 0.223	-0.680 ± 0.127	-0.949 ± 0.147	-1.228 ± 0.095
PC1	k	0.169 ± 0.023	0.020 ± 0.003	0.005 ± 0.002	0.001 ± 0.001
1,800–1,670 cm^{-1}	R^2	0.898	0.862	0.477	0.369
First order	A_0	2.689 ± 0.178	2.703 ± 0.059	2.554 ± 0.119	2.696 ± 0.086
PC2	k	0.218 ± 0.021	0.030 ± 0.001	0.003 ± 0.001	0.001 ± 0.000
1,330–1,127 cm^{-1}	R^2	0.978	0.993	0.698	0.813

A is the response variable (PC1 or PC2) and k is the rate constant (weeks^{-1}). A_0 is the initial value of A . R^2 is the coefficient of determination adjusted for degrees of freedom. Data calculated with averaged values per storage time and temperature (N)

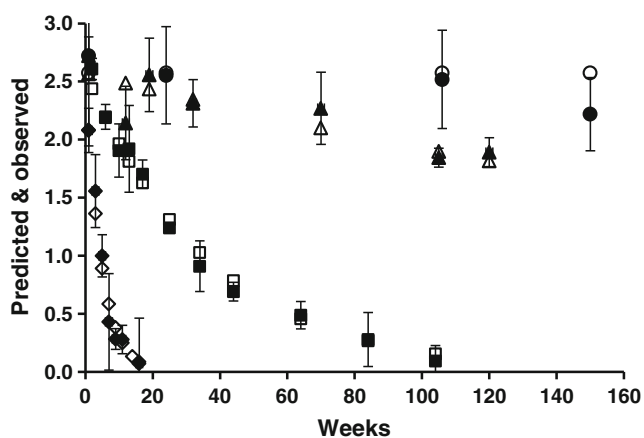


Fig. 4 Predicted (*open symbols*) and observed (mean±standard deviation, *closed symbols*) values for PC2 of the 1,330–1,127 cm^{-1} region, as a function storage time for hake fillets stored at $-10\text{ }^{\circ}\text{C}$ (*diamond*), $-20\text{ }^{\circ}\text{C}$ (*square*), $-30\text{ }^{\circ}\text{C}$ (*triangle*) and $-80\text{ }^{\circ}\text{C}$ (*circle*)

carried information on the time and temperature dependence (Fig. 3) in a more clear way than the individual markers for each region (Fig. 2). The order of reactions for the chosen PCs in each spectral range (Table 2) is in agreement with the expected behaviour for other markers in frozen hake fillets (Sánchez-Valencia et al. 2014) and in general for frozen foods since the majority of the studied food reactions have been characterized as pseudo-zero or pseudo-first-order kinetics (Taoukis et al. 1997). However, the fitting of the 3,100–2,750 cm^{-1} characteristic of the CH stretching vibrations of the methylene and methyl groups in the fatty acid chains was poorer, as expected from previous results (Sánchez-Alonso et al. 2012a). According to Bremner et al. (1987), most deteriorative processes in fish follow similar energies of activation. The models from the three regions of the FTIR spectra (Table 3) were not different to those of Kramer shear resistance reported previously (Sánchez-Valencia et al. 2014), which is an advantage since the use of one marker can estimate the other.

As discussed previously (Sánchez-Valencia et al. 2014), information provided by kinetic modelling can be applied for inspection to monitor temperature exposure, or authentication purposes when mislabeling or fraud is suspected, serve to find time-temperature indicators that correlate best with actual quality changes of the muscle in the studied conditions, or be useful for assuring homogenous products or premium quality ones in the industries. The fact that the evolution with time and temperature of PCs runs in parallel with observed changes in bands attributed mainly to lipolysis (Fig. 2) allows to relating these empirical models with structural data obtained by spectroscopic analysis (Sánchez-Alonso et al. 2012a). The usefulness of these models for estimation of time and temperature or as quality indicators should be validated with new batches of fish, for example, with accelerated shelf life testing.

Conclusions

Kinetic modelling was successfully applied to FTIR spectroscopic data of lipids extracted from frozen stored hake muscle. From the three regions studied, known to carry specific physical chemical information on the changes in fish lipids, the best model was obtained with the spectral data from the 1,330–1,127 cm^{-1} range, corresponding to the PO_2^- group of the phospholipids. The temperature dependency of the rate of reactions, measured by B values, was similar to that previously found for physical chemical parameters well related to sensory quality for this species. This suggests the potential of these markers for the authentication of frozen storage time and temperature and for the estimation of the quality of lean fish flesh.

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