

Low-Field Nuclear Magnetic Resonance of Proton (^1H LF NMR) Relaxometry for Monitoring the Time and Temperature History of Frozen Hake (*Merluccius merluccius* L.) Muscle

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Abstract The objective of the work was to investigate the potential of low-field nuclear magnetic resonance of protons (^1H LF NMR) relaxometry to produce indicators useful in the estimation of the quality and shelf life of frozen hake (*Merluccius merluccius* L.) muscle as affected by storage temperature and time. ^1H low-field NMR T_2 transverse relaxation measurements were performed from fillets of a total of 145 hakes that had been stored at -10 , -20 , -30 , or -80 °C for up to 150 weeks. The principal component (PC) scores from the ^1H LF NMR distribution of T_2 relaxation times were adjusted to zero-order kinetics and the reaction rates followed an Arrhenius-type pattern. Kinetic modeling using the PC scores of the 12–400-ms range resulted in the best model corresponding to both T_{21} and T_{22} regions. The temperature dependency of the rate of reactions, measured by B values, was similar to that previously found for shear resistance and for the hydrolysis of phospholipids, well-known markers related to the sensory quality in this species. Thus, our results suggest that this methodology has a great potential to evaluate the frozen storage time and temperature in hake as well as to provide an indication of the expected quality of the fillet.

Keywords Fish quality · Hake · Frozen storage · ^1H LF NMR · Kinetic modeling · Arrhenius

Introduction

The quality of fish muscle is related to some measurable attributes and properties that can be determined by standard methods which provide indicators that, in turn, can be used to evaluate the product or to set specifications (Bremner 2000) and which may be of different relative importance to the various parts of the supply chain in the fish sector (Jørgensen et al. 2003).

Fish muscle contains proteins, lipids (most notably the omega-3 fatty acids, DHA, and EPA), and a wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium, selenium, and iodine, making it a very good source of high-value nutrients. Fish also have special eating features largely due to the specific nature of the constituent muscle proteins and their structural configurations, as well as the high water holding capacity, that make it so acceptable as a food (Mackie 1997).

Unfortunately, one of the characteristics of fish muscle is its high perishability. Therefore, and in order to minimize spoilage processes, fish is usually stored at low temperatures, either above freezing, if the fish is to be consumed in a short time, or well below freezing for long-term storage. The latter is one of the most effective methods to prolong shelf life since it decreases the rate of many physical and chemical reactions. However, several factors including species, season, fishing, and/or slaughtering methods, *post mortem* handling, freezing, and frozen storage conditions, as well as their interactions, may affect the fish flesh structure, composition, and functionality, all of which can lead to the loss of acceptability by the consumer (Haard 1992).

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Currently, there is a need to identify methodologies to provide new and objective indicators related to the loss of quality attributes caused by prolonged times and/or high temperatures of frozen storage applicable to estimate the quality and shelf life of frozen fish products and to verify the compliance of European Regulations on the provision of food information to consumers [(EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011] and on the common organization of the markets in fishery and aquaculture products [Regulation (EU) No 1379/2013 of the European Parliament and of the Council of 11 December 2013].

Kinetic models have been successfully used to monitor changes occurring due to both storage time and temperature. The Arrhenius equation, for example, has been applied in many food systems and 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; Careche et al. 2014; 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).

On the other hand, spectroscopic techniques are preferred for fish quality and safety evaluation and the analysis of data is usually performed by chemometrics (Cheng et al. 2013). Spectroscopic markers for kinetic modeling of frozen hake muscle (*Merluccius merluccius* L.) have been found by using principal components (PCs) of Fourier transform infrared (FTIR) spectra of the lipid fraction (Careche et al. 2014). Principal component analysis (PCA) is a technique that reduces a data set into a small number of new, non-correlated variables, on each of which, a score for each sample is calculated (Afifi and Clark 1984), and these scores can reveal patterns within a data set.

Low-field nuclear magnetic resonance of protons (^1H LF NMR) has been used in myosystems mainly in the study of meat and meat products (Bertram and Andersen 2004; Pearce et al. 2011) although their application to fish products has increased recently (Erikson et al. 2004, 2012; Aursand et al. 2008, 2009; Gudjónsdóttir et al. 2011, 2013). In model systems with proteins and other biopolymers, as well as in muscle, it has been shown that the transverse relaxation times (T_2) can provide information about the physical-chemical nature and spatial organization of muscle components (Belton 2011; Erikson et al. 2012). We previously had analyzed hake muscle stored at a fixed temperature (i.e., $-10\text{ }^\circ\text{C}$), and T_2 relaxation times provided information related to deterioration due to frozen storage time (Sánchez-Alonso et al. 2012) which suggested that these indicators might be useful for kinetic modeling.

The objective of the present work was to explore the potential of ^1H LF NMR relaxometry to provide indicators useful in the estimation of quality and shelf life of frozen hake (*M. merluccius*) muscle as affected by temperature and time,

which could be associated to quality standards or could be used to establishing the remaining shelf life of hake products, either alone or in combination with other existing methods.

Material and Methods

Fish Samples

Hakes (*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) and visually inspected, and the scales were removed. The fish in *post-rigor* condition were beheaded, washed with iced water to remove blood and rests of viscera, and allowed to drain in stainless steel racks. Then, they were manually filleted (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 (polyamide 20/polyethylene 70, thickness $90\text{ }\mu\text{m}$, Industrias Plásticas Arias S.L., Lugo, Spain), labeled, 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 $7\text{ }^\circ\text{C}$. Fillets of five hakes were stored refrigerated until they were analyzed and the rest were frozen in a blast freezer (RF 201 AB, Fagor, Mondragón, Spain) for 1 h until the fish reached a core temperature of $-20\text{ }^\circ\text{C}$, placed in polystyrene boxes with dry ice, and immediately transported from Puerto de Celeiro to the ICTAN Laboratory in Madrid. They were stored at $-10\text{ }^\circ\text{C}$ (Liebherr model GGPV 5520, Liebherr GmbH Korneuburg, Austria), $-20\text{ }^\circ\text{C}$ (walk in freezer, DWM Copeland model DLL-401), $-30\text{ }^\circ\text{C}$ (Radiber model CVED-310-30, Radiber S.A. Barcelona, Spain), and $-80\text{ }^\circ\text{C}$ (Nuaire model Glacier -86, Nuaire, Inc., USA).

Those stored at $-10\text{ }^\circ\text{C}$ were sampled up to 23 weeks and the ones at -20 and $-30\text{ }^\circ\text{C}$, for up to 104 and 147 weeks, respectively. Fillets from ten frozen fish were kept at $-80\text{ }^\circ\text{C}$ and analyzed after 106 and 150 weeks of storage. For each storage period, fillets from five individuals were subjected to analyses as described below.

Low-Field NMR Analysis

A ^1H LF NMR analyzer minispec mq20 (Bruker Optik GmbH, Germany) with a magnetic field strength of 0.47 T corresponding to a proton resonance frequency of 20 MHz was used for the NMR measurements. Frozen fillets were cut in two, and the dorsal part was allowed to semi-thaw on a cutting board placed on top of a container filled with ice and cut in $1\times 1\times 2\text{-cm}$ parallelepipeds of about 2 g . The samples

were placed in NMR tubes (1.8-cm diameter and 18-cm height) in an ice bath, let to thaw, and transferred to a thermostated water bath until they reached 4 °C. During the measurements, the samples were kept at ~4 °C by using a variable temperature probe head (model PA225) coupled to a thermo Haake® refrigerated circulator C/DC class DC10-K10 (Fisher Scientific S.L., Madrid, Spain). Transverse relaxation data (T_2) were measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with a τ value of 150 μ s. For each sample, 16 scans were acquired at a 2-s interval with a total of 3000 echoes. At least three parallels per fish were performed.

NMR T_2 transverse relaxation data were analyzed by the measurement of biexponentials and the distribution of relaxation times (Sánchez-Alonso et al. 2012). By the first method, the transverse relaxation data were fitted to a biexponential model using the software Bruker the minispec v 1.2.

$$\text{NMR signal} = A_{21}e^{-\frac{t}{T_{21}}} + A_{22}e^{-\frac{t}{T_{22}}}$$

where T_{21} and T_{22} were, respectively, the shorter and longer transversal relaxation components, A_{21} and A_{22} were the corresponding amplitudes, and t is the acquisition time.

In the second method, the relaxation data were treated using the CONTIN regularization algorithm (Provencher 1982a, 1982b) resulting in the corresponding distributions of the relaxation times from the decay curve; this is also part of the software provided with the equipment (CONTIN the minispec v 1.2). Data were analyzed using SPSS software (IBM SPSS statistics version 20, IBM Corporation 2011).

Data Analysis

The number of individuals per storage time and temperature (replicates) was $N=5$. Data from (a) biexponential fitting to the T_2 transverse relaxation decay curves (A_{21} , A_{22} , T_{21} , and T_{22}), (b) principal component (PC) scores from the decay curves, (c) PC scores from the biexponential parameters, and (d) PC scores from the CONTIN distribution function were taken as the response variables for the analyses 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-order kinetics. The equation can be expressed as

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

where A_0 is the value of the response variable at time 0, A is the value of this response variable at time t , and k is the rate constant.

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

$$k = k_A \exp\left(\frac{-B}{T}\right) \quad (2)$$

where k is the rate constant, T is the absolute temperature in Kelvin, k_A is the pre-exponential factor, and B is a constant. Time and temperature can be modeled in one step by using non-linear 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 non-linear regression by using the T_{ref} approach for zero-order kinetics is written as follows:

$$A = A_0 + k_{\text{ref}}t \exp\left[-B\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right] \quad (3)$$

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 °C), close to the maximum recommended storage temperature of −18 °C. Starting values for the non-linear regression were calculated using the parameters calculated by applying Eqs. 1 and 2. For the final model, A_0 , k_{ref} , and B parameters were estimated with all data from −10, −20, and −30 °C ($N=135$ fish) using the Non-Linear Regression program (Levenberg-Marquardt algorithm) with the SPSS software (IBM SPSS statistics version 20, IBM Corporation 2011).

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Results and Discussion

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

Influence of Storage Time and Temperature

Figure 1 shows initial distribution of relaxation times of hake muscle frozen stored at the different temperatures, as well as unfrozen muscle. In the latter case, a major band was observed at relaxation times corresponding to T_{21} and the band corresponding to T_{22} was not present, as it was previously observed for this species (Sánchez-Alonso et al. 2014). In muscle stored at $-80\text{ }^{\circ}\text{C}$, T_{21} band widened and its maximum height decreased as compared to the unfrozen muscle, and no T_{22} band was observed, as in unfrozen muscle. At increasing frozen storage temperatures, a progressive widening and decreasing intensity of the T_{21} band was observed concomitantly with the appearance of the T_{22} population. Intensity peak of the T_{21} band appeared at values corresponding to protons with more restricted mobility as the storage temperature was higher. The differences between frozen/thawed and unfrozen fish were previously observed in an experiment where various freezing rates were studied (Sánchez-Alonso et al. 2014). Following the interpretation given by Belton (Belton et al. 1988; Belton 2011) based in model systems of biopolymers and water, it was suggested that the appearance of T_{22} in a thawed product could mirror the morphological changes resulting from freezing and thawing (Sánchez-Alonso et al. 2014). The absence of this band in $-80\text{ }^{\circ}\text{C}$ frozen samples can be explained if we assume that morphological changes due to the formation of small ice crystals are small at this temperature. Belton et al.

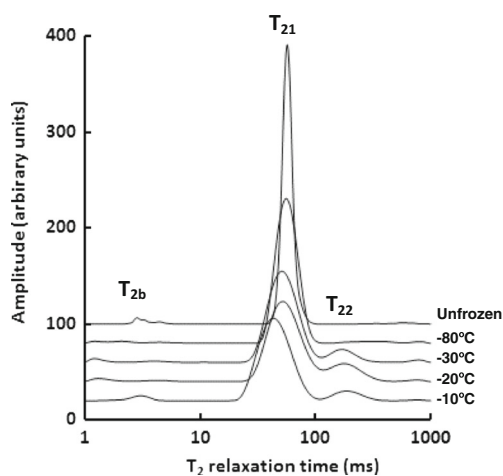


Fig. 1 ^1H LF NMR distribution of T_2 relaxation times of the following: unfrozen hake muscle and hake muscle stored at -10 , -20 , -30 , and $-80\text{ }^{\circ}\text{C}$ for the first storage time analyzed. Numbers at the right side show the storage temperature for each curve

(1988) did not find either any extra band in thawed agarose gels that had been frozen with liquid nitrogen when the size of the ice crystals was smaller than $3\text{--}4\text{ }\mu\text{m}$.

The modifications of the T_2 relaxation time distribution at increasing storage times were more evident with increasing storage temperature (Fig. 2). These changes affected the relative abundances of protons with intermediate (T_{21}) and high (T_{22}) mobility. Relaxation times in the range of $1\text{--}4\text{ ms}$ (T_{2b}) were also observed. The relative abundance of T_{21} decreased with storage time, and a simultaneous increase of T_{22} was observed. Likewise, at higher temperatures and longer storage times, a progressive shift of T_{21} band toward lower relaxation times was found. No clear trend was identified for T_{2b} . The data obtained at $-10\text{ }^{\circ}\text{C}$ were in accordance with previously reported results in hake (Sánchez-Alonso et al. 2012, 2014) and cod (Steen & Lambelet 1997) although, in none of these cases, the storage times covered the range of storage temperatures and times of the present work.

The progressive decrease of T_{21} relaxation time may be explained by a chemical exchange between water and myofibrillar proteins protons affected by denaturation and aggregation (Sánchez-Alonso et al. 2014). This process was explained by Hills et al. (1989), based in relaxometry experiments with bovine serum albumin (BSA): the authors observed lower T_2 transverse relaxation of protons from aggregated BSA than from unfrozen BSA solutions. The increase of T_{22} and decrease of T_{21} abundances along storage time have been suggested to be associated to a migration of intramyofibrillar water to extracellular crystals (Sánchez-Alonso et al. 2014).

T_2 transverse relaxation time parameters extracted by biexponential fitting (Fig. 3) showed, for all four parameters (T_{21} , T_{22} , A_{21} , A_{22}), changes along storage. At -10 and $-20\text{ }^{\circ}\text{C}$, the intermediate relaxation time (T_{21}) and its corresponding amplitude (A_{21}) decreased gradually whereas the relaxation time corresponding to more mobile protons (T_{22}) and its amplitude (A_{22}) increased in these conditions. However, at $-30\text{ }^{\circ}\text{C}$, the variations were less pronounced, and at $-80\text{ }^{\circ}\text{C}$, no changes were observed except for T_{22} .

Thus, large changes were observed in the ^1H LF NMR relaxometry measured values which kept a direct relationship with the storage temperature and time, i.e., the higher the temperature and the longer the time, the larger the change. This suggests the value of these measurements as indicators for frozen storage quality loss.

Kinetic Models

Several kinetic models were calculated using the parameters: a) from exponential fitting to the T_2 transverse relaxation decay curves (A_{21} , A_{22} , T_{21} and T_{22}), b) PC scores from the decay curves, c) PC scores from the biexponential parameters, and d) PC scores from the CONTIN distribution function. Only the later will be described since it gave the best results.

Fig. 2 ^1H LF NMR distribution of T_2 relaxation times of hake muscle stored at -10°C (upper left), -20°C (upper right), -30°C (lower left), and -80°C (lower right) for up to 150 weeks. Numbers at the right side show the storage time in weeks for each curve

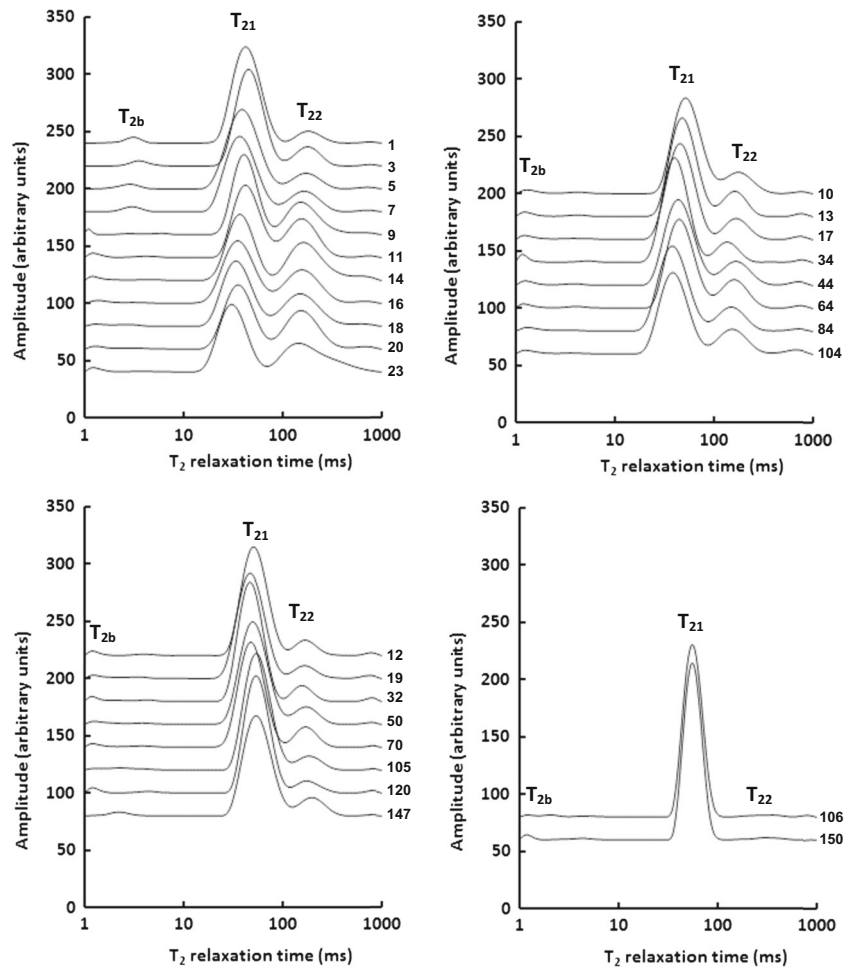
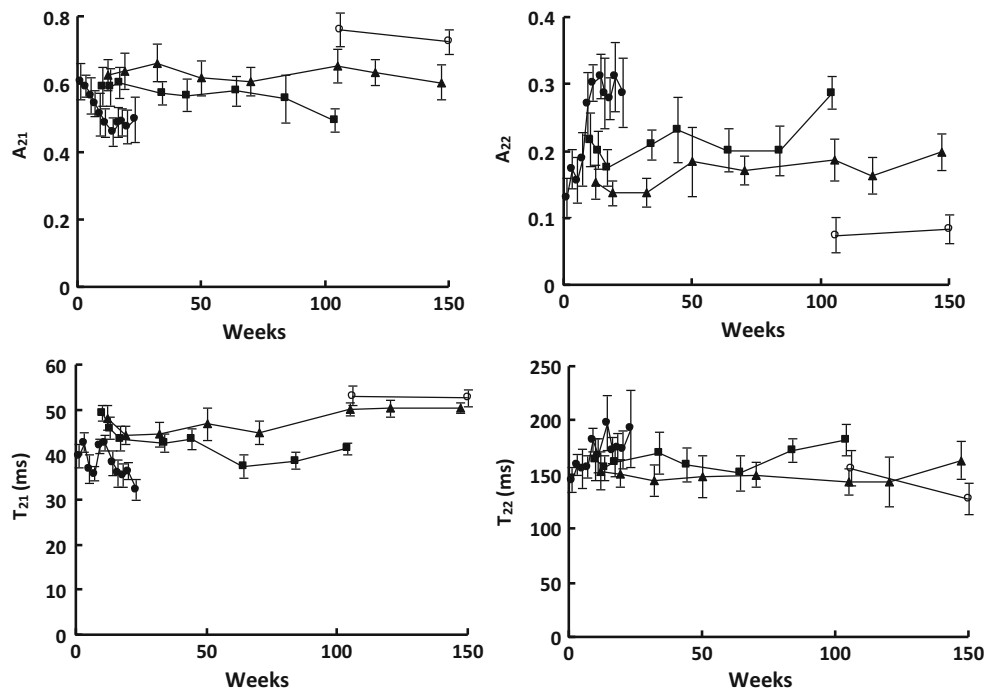


Fig. 3 ^1H LF NMR relaxation parameters (A_{21} , T_{21} , A_{22} , and T_{22}) of hake fillets stored at -10°C (closed circles), -20°C (closed squares), -30°C (closed triangles), and -80°C (open circles). Mean \pm standard deviation $N=145$ fish



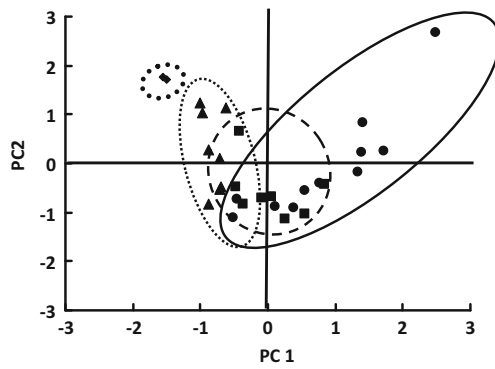


Fig. 4 PCA score plots of T_2 relaxation time distribution (12–700 ms) of hake fillets stored at -10 °C (circles), -20 °C (squares), -30 °C (triangles), and -80 °C (diamonds)

Factor analysis was performed with T_2 relaxation time distribution mean data values averaged by week in the range of 12 and 700 ms. Nine PCs explained the 99.7 % of the variance; PC1 and PC2, which explained 61 and 15 % of the variance, respectively, showed a clear relation with storage temperature (Fig. 4).

The intermediate and long relaxation components might keep a different relation with storage time. In order to check this, we used PCAs from different ranges (12–112; 12–400; 12–700; 112–400; 112–700 ms) of the T_2 relaxation time distribution, thus covering protons with intermediate and long relaxation times separately or in combination. A similar approach was performed for FTIR of the lipid fraction (Sánchez-Alonso et al. 2014) and showed

distinct features depending on the region of the spectra. The first PC of each of the analyzed ranges explained between 60 and 85 % of the variance, and since all these PCs kept a relationship with temperature and time, they were used for kinetic modeling. In all cases, the best R^2 values were found for zero-order kinetics (Table 1). The Arrhenius parameters by non-linear regression analysis (Table 2) showed that the best models were obtained when using ranges where both intermediate and high-mobility protons were included (12–400 and 12–700 ms), in terms of lower-standard errors and higher R^2 . Although differences between both ranges were small, the best one was between 12 and 400 ms. For this range, the final kinetic model was performed with all data values (Table 3, Fig. 5) and as expected, lower-standard-error values were obtained. The residuals were independent and normally distributed, and they had constant variance and zero mean. The model had a good agreement between predicted and observed values at -10 and -20 °C being worse at -30 °C.

Several other methods have been proposed to predict the frozen storage time of hake products at a fixed temperature, including the quality index method (QIM) based on sensory inspection (Herrero et al. 2003), stress relaxation test (Herrero and Careche 2005), visible spectroscopy (Heia et al. 2003), or a combination of methods such as apparent viscosity of muscle homogenates, puncture test, and Kramer shear resistance (Herrero and Careche 2006).

One of the main advantages of ^1H LF NMR, as an indicator of the quality of frozen fish muscle, is to obtain relevant information at the ultrastructural level (heterogeneity in muscle

Table 1 Parameters (\pm standard error) of the regression equations of zero-order ($A_0 - A = kt$) of PC1 scores obtained from selected ranges of the of T_2 transverse relaxation time distribution of hake fillets stored at -10 , -20 , and -30 °C

PC1 scores of T_2 transverse relaxation times	Zero-order kinetics parameters	Temperature (°C)		
		-10 ($N=11$)	-20 ($N=8$)	-30 ($N=8$)
PC1 score from 12 to 112 ms	A_0	-0.350 ± 0.281	-0.790 ± 0.209	-0.796 ± 0.120
	k	0.101 ± 0.021	0.014 ± 0.004	-0.003 ± 0.001
	R^2	0.72	0.72	0.43
PC1 score from 112 to 400 ms	A_0	-0.768 ± 0.345	-0.577 ± 0.283	-1.166 ± 0.170
	k	0.125 ± 0.026	0.012 ± 0.005	0.004 ± 0.002
	R^2	0.73	0.49	0.37
PC1 score from 112 to 700 ms	A_0	-0.904 ± 0.352	-0.613 ± 0.206	-1.012 ± 0.122
	k	0.135 ± 0.026	0.013 ± 0.004	0.002 ± 0.001
	R^2	0.75	0.68	0.22
PC1 score from 12 to 400 ms	A_0	-0.637 ± 0.154	-0.737 ± 0.063	-1.063 ± 0.118
	k	0.124 ± 0.011	0.014 ± 0.001	0.001 ± 0.001
	R^2	0.93	0.96	0.04
PC1 score from 12 to 700 ms	A_0	-0.729 ± 0.200	-0.732 ± 0.056	-0.989 ± 0.101
	k	0.130 ± 0.015	0.014 ± 0.01	0.000 ± 0.001
	R^2	0.89	0.97	0.001

A is the response variable (PC1) 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 (N)

Table 2 Regression parameters (\pm standard error) of the Arrhenius equation for PC1 scores obtained from selected ranges of the of T_2 transverse relaxation time distribution of hake fillets stored at -10 , -20 , and -30 °C

Arrhenius equation parameters	PC1 scores of T_2 transverse relaxation times (range, ms)				
	PC1 (12–112)	PC1 (112–400)	PC1 (112–700)	PC1 (12–400)	PC1 (12–700)
A_0	-0.843 ± 0.129	-0.870 ± 0.142	-0.895 ± 0.129	-0.919 ± 0.076	-0.923 ± 0.080
k_{ref}	0.014 ± 0.003	0.016 ± 0.004	0.017 ± 0.003	0.016 ± 0.002	0.016 ± 0.002
B	15133 ± 1516	14062 ± 1418	13928 ± 1241	14671 ± 770	14508 ± 796
R^2	0.83	0.80	0.83	0.94	0.93

One-step non-linear regression with zero-order kinetics ($A=A_0+k_{ref}t \exp [-B(1/T-1/T_{ref})]$). T_{ref} is the reference temperature set at 253 K. A is the response variable (PC1 scores), A_0 is the initial value of A , k_{ref} is the rate constant (week^{-1}) at T_{ref} , B is a constant (K), t is the storage time (weeks), and T is the absolute temperature (K). R^2 is the coefficient of determination adjusted for degrees of freedom. Data calculated with averaged values per storage time (N)

spaces by the presence of ice crystals) and molecular level (e.g., protein aggregation). This information is consistent with the results obtained by other physicochemical techniques that measure the quality in frozen fish, which in turn are already known to correlate well with other spectroscopic parameters (Sánchez-Alonso et al. 2012). Thus, standardization of the method proposed here could raise the possibility of replacing the physical-chemical techniques for the faster relaxometry ^1H LF NMR methods.

Other indicators such as Kramer shear resistance, water holding capacity, and the FTIR spectra of the lipid fraction of hake muscle have shown to follow an Arrhenius-type equation and could therefore be used to predict time at a given temperature and vice versa (Sánchez-Valencia et al. 2014; Careche et al. 2014). The functions that integrate time and temperature in order to measure quality for processes occurring during storage of fish muscle usually follow a similar pattern (Bremner et al. 1987). The results obtained for parameter B (i.e., temperature dependence of a given reaction rate) with ^1H LF NMR showed similar values to those found using shear resistance (Sánchez-Valencia et al. 2014) and hydrolytic changes in the phospholipid fraction as measured by FTIR spectra of fish lipids (Careche et al. 2014).

Table 3 Regression parameters (\pm standard error) for PC1 scores of the T_2 relaxation time distribution (12–400 ms) of hake fillets stored at -10 , -20 , and -30 °C

PC1 scores (12–400 ms)	Arrhenius equation parameter	Estimation
Zero-order kinetics	A_0	-0.869 ± 0.027
	k_{ref}	0.015 ± 0.001
	B	14690 ± 293
	R^2	0.81

These regression parameters were extracted from the equations of zero ($A=A_0+k_{ref}t \exp [-B(1/T-1/T_{ref})]$) order with a reference temperature (T_{ref}) set at 253 K. A is the response variable (PC1 for each range), k_{ref} is the rate constant (week^{-1}) at T_{ref} , B is a constant (K), t is the storage time (weeks), and T is the absolute temperature (K). A_0 is the initial value of the response variable. $N=135$ fish

The estimation of storage times showed a good fitting at relatively high temperatures (e.g., -10 and -20 °C) (Fig. 5), but at lower ones (e.g., -30 °C), there was a lack of fit between observed and predicted values. This may be, in part, a consequence of the experimental error, but also to a biological zero temperature at which all reactions stop (Bremner et al. 1987). For many reactions in frozen stored hake, this temperature could be close to -30 °C. This would involve a lack of linearity of the activation energies as a function of temperature which could only be achieved in a certain range. In fact, when data at -80 °C were included, the kinetic models worsened (not shown), thus supporting the halting of biological reactions. Models other than Arrhenius have been proposed (e.g., Peleg et al. 2002) for ranges including temperatures where the system may be practically inert, together with temperatures where the activity of the system is accelerated. Nevertheless, and given that the temperatures of interest for

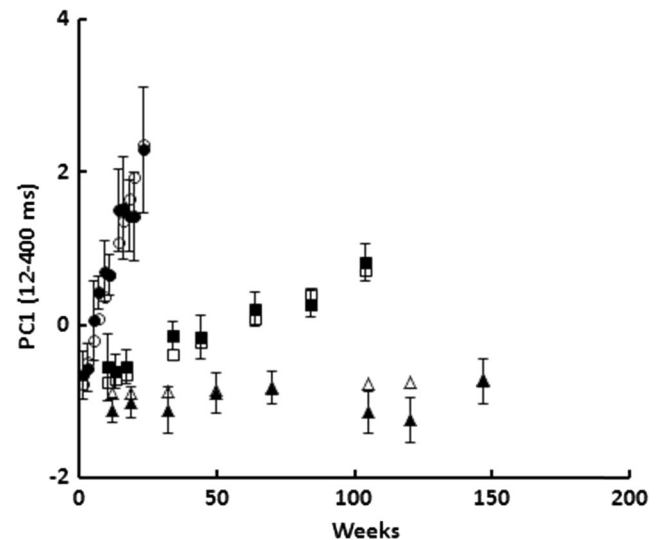


Fig. 5 Predicted (open symbols) and observed (mean \pm standard deviation, closed symbols) values for PC1 scores of the T_2 relaxation time distribution (12–400 ms) as a function storage time for hake fillets stored at -10 °C (circles), -20 °C (squares), and -30 °C (triangles). $N=135$ fish

most fish products are between -10 and -30 °C, we considered that the used Arrhenius in this range would be suitable to estimate the product's quality.

As discussed previously (Sánchez-Valencia et al. 2014; Careche et al. 2014), information provided by kinetic modeling can be applied for inspection or authentication purposes; it could also help in finding time-temperature indicators with close correlations with muscle quality changes. Another advantage would be their usefulness to ensure homogenous or premium quality products in the processing industry. In any case, the usefulness of ^1H LF NMR models for estimation of time and temperature or as quality indicators should be validated with new batches of fish. A similar experimental approach should be used for other species and/or conditions so that a product-specific time temperature relationship could be identified and used for quality monitoring.

Conclusion

Kinetic modeling was successfully applied to ^1H LF NMR data from frozen stored hake muscle. The best model was obtained with PC1 from the spectral data of the 12–400-ms range, corresponding to both T_{21} and T_{22} relaxation times. The temperature dependency of the rate of reactions, measured by B values, was similar to that previously found for shear resistance and changes in the lipid fraction, mainly phospholipid hydrolysis, that are well documented to be related to sensory quality for this species. Accordingly, we suggest that this methodology has a great potential to estimate frozen storage time and temperature which, in turn, could be related to the quality of lean fish flesh under commercially relevant conditions.

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