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ScienceDirect
Journal of Food Engineering 88 (2008) 263–271
JOURNAL OF FOOD ENGINEERING
www.elsevier.com/locate/jfoodeng

Bioelectrical impedance analysis of frozen sea bass (*Dicentrarchus labrax*)

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Received 31 August 2007; received in revised form 29 January 2008; accepted 9 February 2008
Available online 19 February 2008

Abstract

The potential of bioelectrical impedance analysis was investigated to differentiate sea bass samples (*Dicentrarchus labrax*) subjected to different freezing methods and numbers of freezing cycles. The resistance (R) and reactance (X) spectra recorded at 19 frequencies (1 Hz–1 MHz) were analyzed by principal component analysis (PCA). Discriminant analysis (DA) was applied to the first three principal components obtained by PCA, physical and chemical variables. The classification was found to be correct for 78% of the samples. It was observed that reactance (X) measured at frequencies higher than 500 kHz can be used to distinguish sea bass samples with different freezing histories. It was concluded that the most important electrical properties of frozen/thawed fish are extracted at frequencies 500 kHz–1 MHz, so that future studies should focus on the electrical properties measured at these frequencies. Additional statistical analysis (ANOVA) showed that differentiation between once-frozen and twice-frozen samples was more successful than differentiation between fast-frozen and slow-frozen samples.

Keywords: Bioelectrical impedance analysis; Freezing; Fish; PCA; DA

1. Introduction

Due to the complexity of the tissue injury as a consequence of freezing, establishing an efficient method for quality control of frozen fish has long been regarded as a difficult task, and to date there is no entirely satisfactory physical method for evaluating frozen fish quality. As documented before, the most important types of tissue damage during freezing are reflected in a change of tissue impedance (Yu et al., 2004).

Although researchers have been investigating impedance measurements on frozen and/or chilled fish for more than 40 years, the electrical response over an entire frequency region from 1 Hz to 1 MHz has never been investigated. One of the first physical methods used in quality control of fish was to measure electrical resistance at two frequencies in a kHz region with an Interelectron Fish tester (Hennings, 1964). This application of impedance methodology only examined the spectra as a means of choosing optimum frequencies at which to make the measurements. These first investigations resulted in the production of several electric devices –Torrymeter, RT Meter – which have been successfully used as secondary tools in standard veterinary practice for assessing fish freshness. Nowadays, electric devices of this type (in conjunction with several other physical methods) are being used to try and achieve a multi-sensor device for the control of fish freshness (Olafsdottir et al., 2004).

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doi:10.1016/j.jfoodeng.2008.02.010

Recently, Yu et al. (2004) measured impedance of fish fillets at 100 Hz and obtained promising results for different freezing/thawing conditions. The authors measured the impedance of fish samples which were once- or twice-frozen using different freezing and thawing methods.

A number of spectroscopic methods have been used quite recently to measure the quality-determining properties of frozen fish (Karoui et al., 2006; Kent et al., 2004, 2005). A common feature of spectroscopic methods is that they can provide a multivariate result; however, to obtain the final result, measured values have to be transformed with advanced mathematical procedures like principal component analysis – PCA (Jørgensen, 2001). PCA is now recognized as a very useful method for all the multivariate measurement techniques because all the measured information can be extracted in fewer variables. Impedance results have been analyzed in a number of ways: in quality control of fish, the principal method of data analysis has been to calculate indices with the measurements conducted at one or two frequencies (Hennings, 1964; Yu et al., 2004). With living tissues and in post-mortem period, impedance data have been analyzed by regression at each measured frequency and at several selected frequencies, by Cole–Cole analysis, etc. (Thomas et al., 1998), but multivariate techniques of data analysis are still not widely used.

Recently, Kent et al. (2004,2005) measured dielectric properties of frozen fish at microwave frequencies, with subsequent transformation of the spectra using the PCA method. The authors developed a new method for quality control of fish which correlated well with sensory analysis as well as other quality-related attributes. Although the theoretical basis and initial idea came from studies in the audio and radio frequency regions (Hennings, 1964), the electrochemical processes that take place at microwave frequencies are different from the ones that occur at lower frequencies. The basic electrochemical mechanisms that operate at microwave frequencies are dipolar in polar media, such as water, salts and proteins (Grimnes and Martinsen, 2000).

In our study, the impedance of thawed sea bass fillets was measured in a lower frequency region and we assessed whether there were electrical responses by which fish with different freezing histories can be distinguished. Since Kent et al. (2004,2005) reported that these responses exist at high microwave frequencies and Yu et al. (2004) obtained positive results with the measurements at 100 Hz, we collected the electrical information from unfrozen/frozen fish fillets from 1 Hz to 1 MHz and analyzed it using multivariate statistical tools.

2. Materials and methods

2.1. Preliminary experiment

For the preliminary experiment, 30 samples of farmed sea bass (*Dicentrarchus labrax*) were bought from the local fish farm. Average sample weight was 450–500 g. All samples had the same feeding and rearing conditions, and were caught on the same day, then killed, chilled and transported to the Laboratory. After 12 h in chilled storage, samples were filleted and cut to the same sizes, and electrical parameters were measured (details in Section 2.4). After the measurements, the samples were placed in a freezer ($T = -20 \pm 2$ °C). After 14 days, the samples were thawed by air ($T = +4$ °C) and the measurements of electrical parameters were repeated. After these measurements, the same samples were frozen again in the same conditions and thawed after 14 days, and the electrical parameters were measured again.

2.2. The main experiment

The main part of the experiment was conducted on 100 samples of farmed sea bass (*D. labrax*) which were bought from the local fish farm. Samples had the same average weight as in the preliminary experiment and the post-mortem treatment. After 12 h in chilled storage, the fish were filleted and divided into five groups. The first 20 samples of fish were treated as an unfrozen group. 80 fish were divided into four groups and subjected to different numbers of freezing cycles at different freezing rates. The fillets were weighed and placed in a plastic bag prior to freezing in order to minimize dehydration and contact with oxygen during frozen storage. Two different freezing procedures were used: freezing in a freezer with a natural air-flow (slow freezing) and freezing by immersion in liquid nitrogen (fast freezing). The frozen storage period was 14 days at -20 ± 2 °C. Temperature in the freezer during frozen storage was measured by Precision Temperature Logger, EBI-2T-211, Ebro. Thawing was performed by air, at +4 °C, for 12 h. The fish fillets were subjected to electrical, chemical and physical measurements in 5 groups, as follows:

- Unfrozen group (UN): measurements performed 24 h after capture.
- Slow-frozen group (S): slow freezing–frozen storage (14 days)–thawing–measurements.
- Slow-frozen in two cycles (SII): slow freezing–frozen storage (14 days)–thawing–slow freezing–frozen storage (14 days)–thawing–measurements.
- Fast-frozen group (F): Fast freezing–frozen storage (14 days)–thawing–measurements.
- Fast-frozen in two cycles (FII): fast freezing–frozen storage (14 days)–thawing–fast freezing–frozen storage (14 days)–thawing–measurements.

Two additional samples of fish were used to measure the rate of slow freezing and thawing after one and two cycles.

2.3. Methods

2.3.1. Chemical measurements

The moisture content was determined using standard AOAC methodology and fat was measured using a solvent extraction method. These chemical measurements were made to check the homogeneity of the samples and were not expected to change significantly during frozen storage.

2.3.2. Physical measurements

The physical measurements were expected to change during frozen storage. Since the fish in this study was *D. labrax* and the storage period was relatively short, changes in quality were expected to be consequences of protein denaturation. The impedance of muscular tissue is reported to change during frozen storage and the mechanisms that are involved in these changes are mainly connected to the changes in proteins. Therefore, the following methods were applied.

2.3.2.1. Water-holding capacity (WHC). The filter paper press method developed by Grau and Hamm (1953) was used to measure the amount of water expressed from a minced sample (dorsal part of epaxial muscle) kept under pressure. In this method, the amount of water expressed is inversely proportional to WHC.

2.3.2.2. Thaw drip. Samples were weighed before storage. After storage and thawing at +4 °C, the drip in the package was poured off and the fish fillet was blotted with a paper towel and reweighed to determine drip loss. Drip was expressed as percentage of weight before storage.

2.3.2.3. pH. The pH probe was inserted directly into the fish fillets at three different locations and the pH was measured (704 pH Meter, Metrohm, glass electrode 6.0236.100).

2.4. Bioelectrical impedance measurements

The HP LCR-Meter-4284A was used to measure resistance (R) and reactance (X). The electronic device operated on 19 frequencies from 1 Hz to 1 MHz, measuring electrical properties by the constant current method (0.2 mA_{ef}). Measurement was configured in a two-electrode format. The fish samples, each 10 cm × 2 cm × 1 cm (cut from the epaxial muscle), were placed on a wooden isolator, and the electrodes were inserted in the muscle tissue. The distance between electrodes (9 cm), puncturing depth (1 cm) and sample temperature were kept constant and strictly controlled. Measurements were performed on samples taken from the chilled chamber (+4 °C). Temperature sensors (Precision Temperature Logger, EBI-2T-211, Ebro) were inserted in two reference samples and the increment of the temperature during measurements was monitored. The average temperature of the samples was 8 ± 0.5 °C and the temperature did not increase by more than 1.5 °C during the measurements.

Three replicate measurements were performed on each of the 20 fish in every group.

2.5. Statistical analysis

SPSS version 9.0 was used for the statistical evaluation of the results.

Since resistance (and reactance) variables were highly inter-correlated, principal component analysis (PCA) was used to obtain a few orthogonal variables – principal components (PCs). PCs were then used, together with chemical and physical variables, for discriminant analysis (DA). One of the assumptions in DA is that variables are not correlated. In this experiment, WHC and thaw drip were highly inter-correlated, and so, only WHC was used for the purpose of DA. DA is used to classify cases into the values of categorical dependent variables. The purpose of this technique is to predict the membership of an individual to a qualitative group defined as a preliminary. "Leave-one-out" classification was used to validate the method. In this procedure, each case is classified using a discriminant function based on all cases except the given one.

Analysis of variance was performed to test the significance of differences in group means of all the physical, chemical and electrical variables between the unfrozen group and frozen groups (UN vs. F, S, FII, SII), between fast-and slow-frozen groups (F vs. S and FII vs. SII) and between once- and twice-frozen groups (F vs. FII and S vs. SII). Univariate contrasts were used to test differences in physical and chemical parameters between the unfrozen (UN) and frozen groups (F, S, FII, SII). To test differences in resistance and reactance measured at 19 frequencies between the unfrozen (UN) and frozen groups (F, S, FII, SII), 19 × 5 scheme was used where "frequency" was within-subject variable, and "group" was between-subject variable. Since interaction between variables was significant, pairwise comparisons were performed to determine which of the frozen groups (F, S, FII, SII) was significantly different from the unfrozen group (UN) at the measured frequencies.

To test the significance of differences in means of physical and chemical variables between fast- and slow-frozen groups (F vs. S and FII vs. SII) and once- and twice-frozen groups (F vs. FII and S vs. SII), a 2 × 2 scheme was used where "rate of freezing" and "number of freezing cycles" were between-subject variables. When interaction between variables was significant, pairwise comparisons were performed for two levels of each variable. To test the significance of differences in means of resistance and reactance between fast- and slow-frozen groups (F vs. S and FII vs. SII) and once- and twice-frozen groups (F vs. FII and S vs. SII), a 2 × 2 × 19 scheme was used where "rate of freezing" and "number of freezing cycles" were between-subject variables and "frequency" was a within-subject variable.

Since interactions between variables were significant, pairwise comparisons were performed.

3. Results and discussion

3.1. Preliminary experiment

Results of the preliminary experiment are presented in Figs. 1 and 2. The purpose of this part of the study was to gain a general idea of the influence of the number of freezing cycles on muscle tissue; in this case no statistical analyses were performed. Because freezing changes the internal structure of biological materials, the measurements on the samples before freezing were treated as the "reference" measurements. Fig. 1 shows the resistance of the samples as a function of frequency: the resistance curve of the samples before freezing decreases linearly with increasing frequency, while the samples after the freezing are relatively unaffected by frequencies higher than 1 kHz. At any frequency from 0.01 to 100 kHz, the resistance decreases with each freezing cycle.

Fig. 2 shows reactance decreasing with frequency at lower frequencies; however, from 10 kHz upwards reactance is independent of frequency for all the given conditions (unfrozen, one cycle of freezing or two cycles of freezing). At most of the frequencies, reactance after two cycles of freezing is the same as after only one cycle, except within a narrow range of high frequencies (Fig. 2).

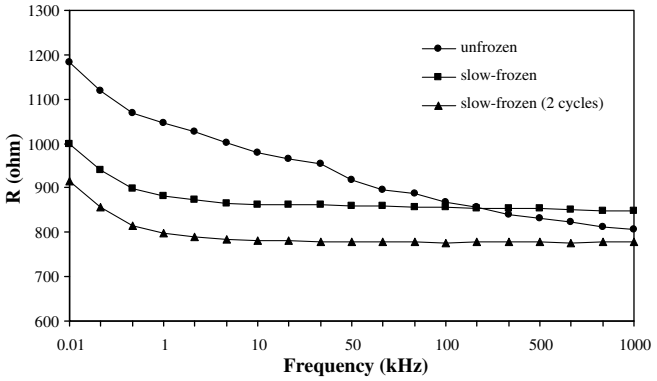

Fig. 1. Resistance spectra of sea bass samples – preliminary experiment.

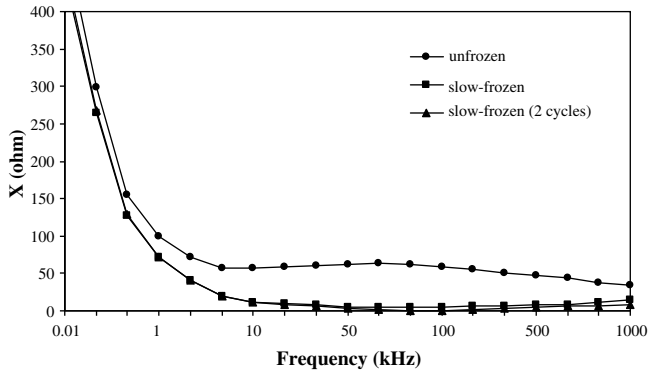

Fig. 2. Reactance spectra (reactance measurements are presented as absolute values) of sea bass samples – preliminary experiment.

In order to provide a better understanding of the extent of the damage to the samples, Yu et al. (2004) presented the results of their measurements (R component measured at frequency 100 Hz) by indices (ratios) between the "reference" measurements and the measurements after one cycle of freezing/thawing, and between measurements after one and two freezing/thawing cycles. The results presented in this way showed the percentage of tissue damage after each cycle of freezing at a given frequency. For different combinations of freezing rates and air thawing, the damage to the fish muscle after one cycle ranged from 20.61% to 41.84%. The second freezing cycle affected the structure less (ratios were 3.61–13.54%), and the authors concluded that damage was mostly caused by the first freezing–thawing cycle and additional cycles hardly affected the structure and properties of the tissue. Our results (100 Hz, resistance component) show that the damage after the first cycle was 15.4%, and after the second cycle 8.9% (calculations are not presented here). Although the level of damage after the second cycle was less than after the first cycle, our results do not suggest that the second cycle has no bearing on tissue damage.

Figs. 1 and 2 show the same shape of the curves after one and two freezing cycles, but at some frequencies the values of resistance and reactance decrease as the number of freezing cycles increases. The difference between the numbers of freezing cycles is visible at most of the frequencies in Fig. 1 and at high frequencies in Fig. 2.

Overall, the preliminary results were encouraging in the sense that the number of freezing cycles could be determined by impedance measurements, and they indicated that both resistive and reactive components might be important for distinguishing fish with different freezing histories, but in a different frequency region.

3.2. The main experiment

The study was designed to evaluate the potential of bioelectrical impedance in two issues: discrimination between freezing rates and discrimination between numbers of freezing cycles. Recently, dielectrical measurements on thawed fish (dielectric time domain reflectometry) and a PCA method of data analysis have shown their potential in prediction of storage temperature and time, and also in discrimination between once- and twice-frozen fish (Kent et al., 2004).

3.2.1. PCA and DA

In our study, PCA was performed with 38 electrical variables (resistance measurements at 19 frequencies and reactance measurements at 19 frequencies) recorded on 100 samples (20 samples per group). The results are shown in Table 1. Three principal components were extracted from the whole spectra (the "Kaiser criterion" was used to deter-

Table 1
Principal component analysis (PCA) of the resistance (R) and reactance (X) spectra measured at 19 frequencies (1 Hz–1 MHz) on sea bass samples subjected to different freezing conditions

Extracted PCs	% of variance extracted by a PC	Original variables extracted in PC ("loading" of PC)
1	75.71	R (0.1–20 kHz); X (2–1000 kHz)
2	18.50	R (50–1000 kHz)
3	5.43	X (0.1–20 kHz)
4	0.23	
5	0.10	

mine the number of PCs). PC1 accounted for 75.7% of total variance and was loaded with the resistance measured at 0.1–20 kHz and reactance measured at 2–1000 kHz. PC2 accounted for 18.5% of total variance and was loaded with the resistance variables measured at 50–1000 kHz. The "loadings" in the SPSS program are correlation coefficients between the measured variables and PCs: when an original variable had a high correlation coefficient with a certain PC, it was assumed that that PC was loaded with that variable.

Discriminating analysis (DA) was performed in order to classify 100 samples into five groups and to determine which of the variables was most responsible for the classification. The following discriminating variables were used: three PCs obtained by PCA, water and fat content, WHC and pH. Four discriminant functions were statistically significant (results are not presented). Results of DA are shown in Tables 2 and 3 and Fig. 3. The first discriminant function was most responsible for the classification of the samples (with the 94.1% of total variance) and presented the highest positive correlation with PC1 (Table 2). WHC presented the second best correlation with it, with a correlation coefficient of 0.29. Fig. 3 clearly shows the differentiation between unfrozen and frozen samples. The results of the classification in Table 3 show that 78% of the samples were correctly classified after cross-validation. All the unfrozen samples were correctly classified, while the classification of the frozen/thawed samples was less successful.

Table 2
Discriminant analysis (DA): correlation coefficients between discriminating variables and discriminant functions

Discriminating variables	Discriminant functions			
	First (94.1%)[a]	Second (4.3%)[a]	Third (1.1%)[a]	Fourth (0.5%)[a]
PC1	0.86	−0.04	0.17	0.39
pH	0.05	0.61	−0.25	0.17
Water	−0.03	−0.01	−0.04	0.07
PC3	−0.01	0.14	0.94	0.03
Fat	0.05	−0.03	0.23	0.13
WHC	0.29	0.36	−0.15	−0.76
PC2	−0.01	0.43	0.03	0.57

[a] % of variance of the discriminant function.

3.2.2. Analysis of variance – chemical and physical variables

In order to better explain the results produced by multivariate techniques, Tables 4 and 5 present mean values of physicochemical and electrical measurements for five groups of fish samples.

The results of the chemical measurements in Table 4 show that the groups do not differ significantly in fat and water content, which was expected since the homogeneity of the samples was controlled. The homogeneity of the samples was the reason for the low correlation coefficients between the first discriminant function and the chemical measurements (Table 2). Kent et al. (2004,2005) have emphasized the importance of sample homogeneity for dielectric measurements when differentiation of fish with different freezing histories is required.

The denaturation of fish muscle proteins during frozen storage produces a change of WHC and a dry, firm and tough texture if the time-temperature profile of storage is unfavorable. WHC is often measured to determinate the quality attributes of frozen/thawed fish. Table 2 shows that WHC is the second most important variable for classification in this work, although the relatively low correlation coefficient (0.29) indicates that samples cannot be properly differentiated using this variable.

Theoretically, bioelectrical measurements are influenced by sample pH. Table 2 shows that pH value was not an important variable in this study, because the pH of the unfrozen group did not differ significantly from any of the frozen groups (Table 4).

3.2.3. Analysis of variance – electrical variables

Table 5 shows the results of ANOVA for electrical variables at six representative frequencies. The results are presented as percentages (indices) indicating the level of tissue damage after one cycle of freezing, after two cycles of freezing and after fast freezing with respect to slow freezing.

The resistance component generally arises from the collision between the current carrying charged particles and the internal structure of the conductor. The intra- and extra-cellular environments of muscle tissue consist mainly of electrolytes (small ions – Cl^-, K^+, Na^+) and have primarily resistive properties (Pliquett et al., 2003). The muscle tissue cells are surrounded by an insulating cell membrane, which is semi-permeable to certain ions. Because of this property the membrane behaves like a leaky capacitor and renders the impedance more reactive. Protein denaturation also alters the electrical response of the tissues.

Freezing, frozen storage and thawing influence the factors cited as responsible for the outputs of resistance and reactance measurements (drip loss, protein denaturation and destruction of membrane), which is the theoretical basis for the use of impedance measurements in quality control of frozen fish.

In light of the results of the preliminary experiment, we expected resistance in the main experiment to decrease with every freezing cycle. Yu et al. (2004) measured resistance at

Table 3
Classification results of discriminant analysis (DA)

Original/cross-validated group	Group of samples[a]	Number of correctly classified samples[b]	Number and groups where there were incorrectly classified samples	
Original	UN	20	0	–
	S	14	6	(1-SII, 5-F)
	SII	18	2	(1-F, 1-FII)
	F	17	3	(2-S, 1-FII)
	FII	14	6	(3-S, 3-SII)
Cross-validated	UN	20	0	–
	S	12	8	(1-SII, 6-F, 1-FII)
	SII	17	3	(1-F, 2-FII)
	F	16	4	(3-S,1-FII)
	FII	13	7	(3-S, 3-SII, 1-F)

UN: unfrozen group.
S: slow-frozen group.
SII: slow-frozen group (two cycles).
F: fast-frozen group.
FII: fast-frozen group (two cycles).
[a] There are 20 samples in every group.
[b] 83.0% of original grouped cases correctly classified; 78.0% of cross-validated grouped cases correctly classified.

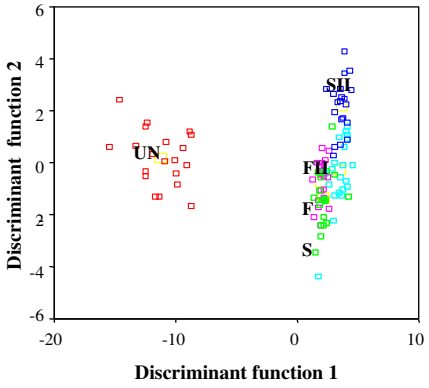

Fig. 3. Results of classification of the sea bass samples in a biplot of the first two discriminant functions.

100 Hz and reported smaller values of resistance after 2 freezing cycles as compared to only one cycle. However, we found no such pattern in our experiment at 100 Hz. The results of ANOVA indicate that there was no significant difference between S and SII (Table 5: 100 Hz, S/SII = 6.67%). Also, the results for fast-frozen samples at 100 Hz in component *R* show that FII suffered less damage than F. The percentage differences in damage between unfrozen and once-frozen groups were 42% (UN/S) and 56% (UN/F) of damage between unfrozen and once-frozen groups, which is similar to the results reported by Yu et al. (2004).

Table 5 shows that reactance measured at 500 kHz and 1 MHz is the only variable that can differentiate between five groups of samples. The reactance measurements for slow-frozen and fast-frozen group were significantly different, although S presented less damaged tissue than F; this is not consistent with the results for WHC and thaw drip.

3.2.4. Distinguishing the groups by bioelectrical impedance analysis

Bioelectrical properties of the frozen materials are different from the unfrozen ones. Yu et al. (2003) showed that temperature/time profile of the pork muscle samples during freezing under −100 °C and thawing by air, is reflected in impedance of the samples (resistance measured at frequency 100 Hz). The strong response in resistance was evident when the samples were going through the phase change. When the samples began to freeze and temperature of the samples stayed for several seconds at

Table 4
Results of chemical and physical measurements (mean values ± standard deviation) of sea bass samples

	Water content (%)	Fat content (%)	WHC (%)	Thaw drip (%)	pH
UN	*76.1 ± 2.86*	*2.3 ± 0.30*	*6.4 ± 1.11*[a]	*0*[a]	*6.4 ± 0.3*
S	75.8 ± 2.89	2.4 ± 0.28	18.8 ± 1.21[c]	5.7 ± 0.92[c]	6.5 ± 0.10[b,c]
SII	75.2 ± 3.23	2.6 ± 0.17	23.2 ± 2.03[b,c]	12.4 ± 2.01[b,c]	6.7 ± 0.16[b,c]
F	75.6 ± 2.44	2.5 ± 0.23	17.4 ± 1.12	4.6 ± 0.33	6.4 ± 0.02[b,c]
FII	75.7 ± 3.18	2.6 ± 0.24	18.1 ± 1.89[b]	5.2 ± 0.41[b]	6.5 ± 0.16[b,c]

Legend as in Table 3.
[a] Expresses a significant difference (ANOVA; $p < 0.05$) between unfrozen and all the frozen groups.
[b] Expresses a significant difference (ANOVA; $p < 0.05$) between the fast- and slow-frozen groups (for S vs. F and/or for SII vs. FII).
[c] Expresses a significant difference (ANOVA; $p < 0.05$) between once- and twice-frozen groups (for F vs. FII and/or S vs. SII).

Table 5
Percentage of tissue damage calculated for six selected frequencies

Frequency	(UN − S)/UN	(S − SII)/S	(UN − F)/UN	(F − FII)/F	(F − S)/F
Resistance (%): $(R_1 - R_2)/R_1 \times 100$					
100 Hz	42.00[a]	6.67	56.23[a]	−19.93[a]	−32.51[a]
1 kHz	55.44[a]	−7.98	58.18[a]	−8.21	−6.56
15 kHz	43.07[a]	−19.56[a]	39.82[a]	−7.58	5.40
50 kHz	32.40[a]	−21.47[a]	27.39[a]	−7.90	6.89
500 kHz	16.70	−22.71[a]	10.11	−8.83	7.33
1 MHz	13.96	−22.49[a]	7.53	−9.24[a]	6.96
Reactance (%): $(X_1 - X_2)/X_1 \times 100$					
100 Hz	0.48	3.56	38.52[a]	−76.91[a]	−61.89[a]
1 kHz	37.40[a]	10.45	62.03[a]	−51.70[a]	−64.85[a]
15 kHz	75.86[a]	19.89[a]	85.15[a]	−22.90	−62.59[a]
50 kHz	83.45[a]	21.15[a]	89.10[a]	−8.76	−51.82[a]
500 kHz	81.16[a]	19.53[a]	82.72[a]	26.45[a]	−9.03[a]
1 MHz	71.02[a]	22.51[a]	72.84[a]	31.84[a]	−6.70[a]

Legend as in Table 3.
[a] Statistically significant difference ($p < 0.05$).

about 0 °C, the resistance increased slowly. After the phase change, when the temperature inside the samples began to decrease quickly, the resistance also quickly increased reaching 100 MHz. During thawing, resistance quickly dropped up to about −40 °C, which was the temperature of melting of the intracellular ice. With further increase of temperature, the thawing process was much slower, that being reflected in the slow increase of resistance.

It is not clear how the impedance (resistance or reactance) during freezing and thawing affects the final impedance of thawed biological materials. Yu et al. (2004) showed that there was no difference in temperature/time curves between one and two cycles of freezing and thawing (freezing with liquid nitrogen, $T = -100$ °C, thawing by air at +20 °C). Consequently, during freezing and thawing, there were no differences in the resistance curves between two cycles.

Our measurements of freezing and thawing rates showed the expected behaviour which was not different when two cycles were compared. (Fig. 4a). During freezing, as phase change occurred and crystallization released a certain amount of heat, temperature became constant with time during a short period around 0 °C. Thawing was quick in the beginning of the process since the first to melt was extracellular ice. As the temperature increased, the thawing process was getting slower.

Although the temperature/time curves during freezing and thawing did not give the explanations for the final differences in bioelectrical properties of the thawed materials, our results indicated that there was a difference in bioelectrical properties of the thawed fish muscle after two cycles of freezing/thawing with respect to one cycle. Yu et al. (2004) found less differences in bioelectrical properties of fish when once- and twice-frozen fish were compared. Other studies have reported small (Hurling and McArthur, 1996; Schubring, 2000) or large (Thiemig and Oelker, 1999) differences in quality between once- and twice-frozen fillets depending on fish species and processing conditions. However, our results still discriminated better on the basis of the number of freezing cycles than the freezing method used. The bioelectrical properties were different when slow-frozen/thawed and fast-frozen/thawed samples were compared, but the results indicated that slow freezing had damaged the structure less than fast freezing, which was not expected.

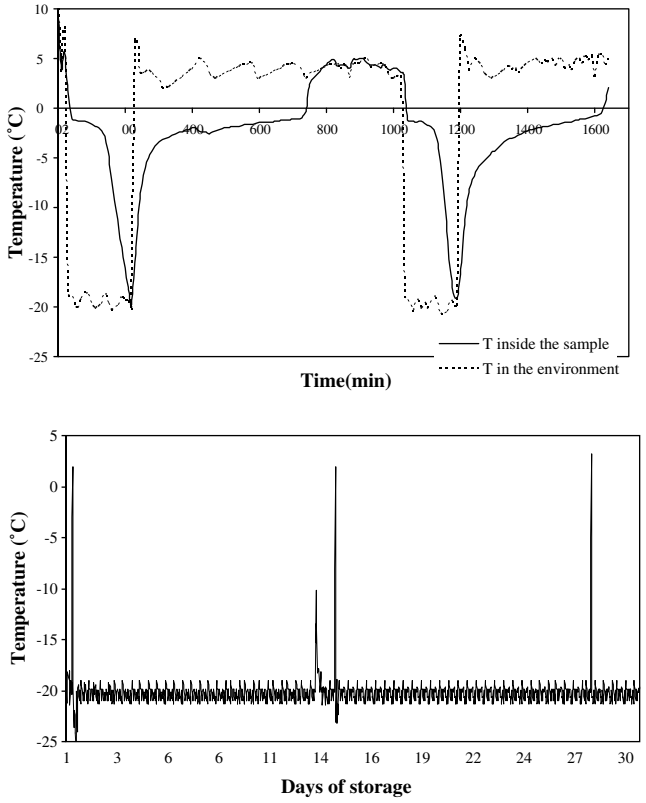

Fig. 4. Temperature and time profile during two cycles freezing and thawing of sea bass fillets (T freezing: 20 ± 2 C; T thawing: +4 ± 1 C) (a). Temperature of the freezer during 30 days of frozen storage (b).

Slow freezing has been reported to cause increased drip loss, and initial advantages of using fast freezing are reported (Chen and Pan, 1997; Pan and Yeh, 1993), although, in other studies of fast freezing, no advantage was observed (Cutting, 1977). However, in quality control of fish, it is been recommended that freezing should be fast. The subsequent frozen storage should follow the temperature of freezing, because advantages gained initially through fast freezing may be lost due to ice crystal growth during subsequent frozen storage at higher temperatures (re-crystallization). However, some studies have shown that for a shorter storage period (up to 2 months), fast freezing (liquid nitrogen) and storage at −20 °C is still a better way for maintaining the initial quality of fish muscle than freezing and storage at −20 °C (Chen and Pan, 1997) In our study, initial advantages of fast freezing may have been lost during frozen storage at -20 ± 2 °C (Fig. 4b). Fig. 4b shows the temperature in the freezer during frozen storage. The sudden increases of temperature on the 1st, the 14th and the 28th day are the consequences of removing the samples from the freezer. The assumption of the occurrence of re-crystallization during frozen storage is supported by the classification results in Table 3, which shows how six out of eight incorrectly classified samples from S group were classified in the F group. Also, three out of four incorrectly classified samples from F were classified in the S group.

Since freezing and thawing of biological materials continue to reflect their changes in impedance (resistance), it is worth noting that re-crystallization might be detected in changes of impedance. The future research will try to address whether re-crystallization affects the impedance response during frozen storage.

Overall results showed that reactance measured at higher frequency is the only variable that can distinguish all the groups of our samples. Why should the imaginary part of impedance have proven to be a more precise indicator of freezing history than the real part? There are possible explanations in recent studies on dielectrical properties of foods. Various factors can influence the electrical properties of foods, including moisture, salt, frequency, temperature and the physical state of foods. The influence of water and salt content depends to a large extant on the manner in which they are bound or restricted in their environment (Ahmed et al., 2007). Freezing causes protein denaturation, which changes the WHC of the fish muscle and causes drip loss after thawing. With drip loss, some liquid is lost, together with some ions. Ahmed et al. (2007) have studied the dielectric response of soy protein solutions, and report that the imaginary part is more sensitive to changes in ionic mobility than the real part. Similar observations have been made by Bircan and Barringer (2002) regarding meat proteins. This might account for our impedance results; since ionic mobility, state and/or concentrations of water and salt are influenced by freezing, it might be that reactance is more sensitive to these changes than resistance.

Electrical properties of different tissues can be measured and described in different frequency regions. The frequency dependence (dispersion) corresponds to specific electrochemical processes. Three regions are of special interest, called α- (characteristic frequencies are in a region mHz–kHz), β- (0.1–100 MHz), and γ-dispersion (0.1–100 GHz) (Foster and Schwann, 1989). Detailed information on dispersion mechanisms can be found in Damez et al. (2007) and Grimnes and Martinsen (2000). Generally speaking, electrical properties of muscular tissue in early post-mortem are measured in a lower frequency region corresponding to α- and β-dispersions. Martinsen et al. (2000) measured the electrical properties of haddock muscle from 1 Hz to 100 kHz as a function of time after the fish was sacrificed, and found that α- and β-dispersions disappeared after the post-mortem period. These changes of electrical properties are associated with the transformation of the tissues from the living (wet) state to the dead (dry) state. The results showed that after the post-mortem period, fish muscle tissue presented no electrical mechanisms significant enough to be measured by impedance in this lower frequency region. However, when the results of our research are placed in an R–X diagram, the difference between the electrical behaviour of unfrozen and frozen/thawed groups is clearly visible. Martinsen et al. (2000) reported an almost linear relationship in an R–X diagram for haddock muscle after the post-mortem period. In our own research the results were the same as the ones reported by Martinsen et al. (2000) when resistance and reactance were measured 24 h after death and placed in R–X diagram (for unfrozen group). With frozen samples, the results were different. Resistance was linear, but reactance presented a peak value (Fig. 5). The peak value was around 500 kHz for all freezing conditions, which could indicate some different electrical property of frozen/thawed tissue with respect to unfrozen tissue. As noted earlier, the protein response in the frequency region 0.1–100 MHz (dispersion) was to be expected and might account for the observed difference between the curves in our study (denaturation of proteins during frozen storage). However, it is not the purpose of this study to explain the differences in the curves, and fur-

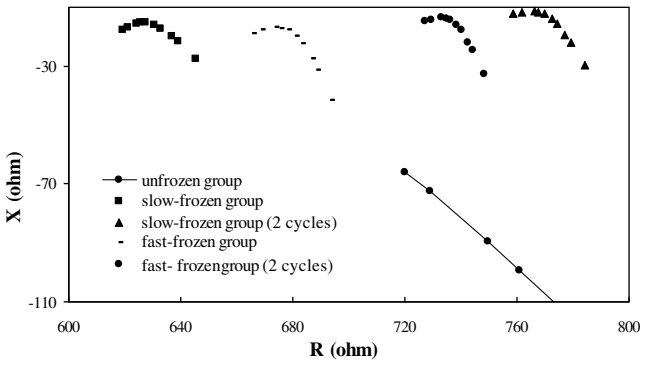

Fig. 5. Resistance and reactance diagram of sea bass samples at higher frequencies (R–X diagram).

ther research is needed to identify the mechanisms responsible for these differences.

If we compare our results with those produced by measuring dielectric properties (Kent et al., 2004), we find that impedance variables are more inter-correlated than dielectric variables (impedance 3 PCs; dielectric variables 10 PCs). These results would suggest that more important electrical information can be extracted at higher frequencies, and that there is no need to use the whole kHz frequency range in further research.

4. Conclusions

All in all, electrical measurements have a potential to differentiate unfrozen from frozen sea bass samples: impedance analysis may be used to differentiate between unfrozen and frozen samples. Imaginary part (reactance) can be used to differentiate between once- and twice-frozen fish as well as between slow- and fast-frozen fish at frequencies higher than 500 kHz, which means that sea bass samples with different freezing histories can be distinguished. From the results of this work it is clear that the important impedance variables are the ones close to MHz frequencies (higher than 500 kHz), which helps to explain why frozen/thawed fish fillets present greater electrical properties in a higher frequency region. However, this experiment was conducted with only one fish species; homogeneity was controlled, and the samples were previously grouped. For a more general view of the use of impedance in quality control of frozen fish, further research is required.

In this study the difference between slow- and fast-frozen samples became lost in the course of frozen storage, which could explain why only 78% of samples were correctly classified. If the frozen storage temperature were properly adjusted for fast- and slow-frozen samples, the percentage of correct classification might be improved.

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