INFLUENCE OF DIFFERENT FREEZING REGIMES ON BIOELECTRICAL PROPERTIES OF ATLANTIC CHUB MACKEREL (SCOMBER COLIAS)

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ABSTRACT

Resistance and reactance of chilled, frozen-thawed and double frozen-thawed fillets of Atlantic chub mackerel (Scomber colias) were measured in a frequency range from 0.1 to 1,000 kHz. After the measurements in the fresh state, one group of samples was slowly frozen in a freezer set at temperature –20 ± 2°C, while the second group was fast frozen by immersion in liquid nitrogen. After 14 days of frozen storage, the samples were thawed and electrical parameters were measured. The measurements were repeated on double frozen-thawed samples. Results showed that the reactance measured at frequencies higher than 150 kHz can distinguish fast frozen from slow frozen fillets. Obtained difference between freezing cycles measured with the reactance at low frequencies could have been influenced by electrode polarization and the consequence of the measuring setup. Overall findings are in accordance with the ones obtained on the farmed fish indicating that the reactance may point to the differences in a fish muscle structure caused by different freezing history.

PRACTICAL APPLICATIONS

This preliminary work is addressing the problem of varying quality of frozen fish fillets on the market today. Therefore, bioelectrical impedance measurements were used to monitor fish tissue properties after freezing under different conditions. Impedance measurements and especially reactance show the potential of detecting the differences among fillets with different freezing history. With further adjustments of the measuring setup and proving the advantage of this method over other spectroscopic techniques, this method may be used in a quality control of frozen fish.

INTRODUCTION

Electrical impedance is the property of a material to resist electrical current flow, and includes both resistance (real part, R [Ω]) and reactance (capacitive, imaginary part, X [Ω]). Impedance can be presented by the equation $Z = R + iX$; the real part $\text{Re}(Z) = R$ is an indication of the ability of the molecules to store electrical energy, and the imaginary part $\text{Im}(Z) = X$ to dissipate it.

Biological tissues are built of structures that have both resistive and capacitive properties (Aberg 2002) that are changing after death of animal and during post-mortem period. The first studies in the field of post-mortem changes in electrical parameters of fish resulted in the production of several electric devices which have been successfully used as secondary tools in standard veterinary practice for assessing fish freshness (Hennings 1964).

Impedance properties of frozen-thawed meats and fish have been studied over the past 40 years. It was published that the observed increase in conductance (i.e., the inverse of impedance) of thawed compared with fresh meats resulted from alterations of the insulating properties of cellular membranes and from modifications of its structure, allowing a greater mobility of ions (Charpentier et al. 1972). However, newer studies stated that meat aged for a very long time can present similarly low impedance as the thawed one, so
that the method would not be appropriate for the discrimination between frozen-thawed and fresh meats (Damez et al. 2008).

On the contrary, studies on the frozen-thawed fish have shown clear difference between frozen-thawed and fresh fish in the bioelectrical properties (Kent et al. 2004, 2005; Yu et al. 2004; Vidacˇek et al. 2008a,b). Recently, impedance of fish fillets at 100 Hz was measured and results showed that fish which had been frozen-thawed in two cycles demonstrated different bioelectrical properties than after only one cycle (Yu et al. 2004). Other authors measured dielectric properties of frozen-thawed fish at microwave frequencies and developed a new method for quality control of fish which correlated well with sensory analysis as well as other quality-related attributes. Because the bioelectrical properties depend on many factors, i.e., chemical composition or state of foods, the authors pointed out that the chemical homogeneity of fish is important in order to obtain positive results on structure differences (Kent et al. 2004, 2005). In our previous studies, resistance and reactance of frozen-thawed sea bass and rainbow trout in a frequency range 1 Hz–1 MHz were measured. To assure the homogeneity of the samples, the studies were done on farmed fish with the same muscle composition and dimensions. The results revealed that the reactance higher than 500 kHz could differentiate the fish with different freezing history (Vidaˇcek et al. 2008a,b).

The aim of this study was to test if frozen-thawed, commercially important wild fish (Atlantic chub mackerel) has similar bioelectrical behavior as the farmed species.

**MATERIALS AND METHODS**

**Experimental Procedure**

Thirty samples of wild Atlantic chub mackerel (Scomber colias) were bought from the local fish market. Average sample weight was 350 ± 15 g. All samples were bought on the same day, chilled and transported to the laboratory. The fish were eviscerated, filleted and divided in two groups (15 samples per group). The epaxial muscle of one fillet of each fish was cut to a size of 10 × 1 × 2 cm and was used for the measurements of electrical parameters. After the measurements in the fresh (unfrozen state), the first group of samples was frozen in a freezer with a natural air-flow at temperature −20 ± 2°C (slow freezing), while the second group was frozen by immersion in liquid nitrogen at temperature −196°C (fast freezing). The frozen storage period was 14 days at −20 ± 2°C for both of the groups. Temperature in the freezer during frozen storage was measured by Precision Temperature Logger, EBI-2T-211 (Ebro, Ingolstadt, Germany). 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 when the electrical measurements were recorded again.

The other fillet of each fish was used for pH measurements, for determination of moisture content and water-holding capacity (WHC). These fillets were also divided in two groups, frozen, stored and thawed as the ones that had been used for the measurements of the electrical parameters.

**Bioelectrical Measurements**

The HP LCR-Meter-4284A (Hewlett Packard, Palo Alto, CA) was used to measure resistance (R) and reactance (X). The electronic device operated on 19 frequencies from 1 Hz to 1 MHz (0.1, 0.2, 0.5, 1, 2, 5, 10, 15, 20, 50, 80, 100, 200, 300, 4,000, 500, 800 and 1,000 kHz), measuring electrical properties by the constant current method (0.2 mA). Measurement was configured in a two-electrode format. The fish samples 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, Ingolstadt, Germany) 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. Measurements were performed in triplicates on each sample.

**Physical and Chemical Measurements**

The moisture content was determined using standard AOAC (1995) methodology.

For the determination of WHC, the filter paper press method was used to measure the amount of water expressed from a minced sample (dorsal part of epaxial muscle) kept under pressure. In short, 2 g of muscle tissue were placed on a Whatman no. 1 filter paper (which was weighted previously) and pressed between two glass plates by a weight of 200 g for 15 min. The water which was squeezed out was absorbed by the filter paper. WHC is obtained by the difference of the weight of the filter papers after and before measurements and is related to the weight of the sample. In this method, the amount of water expressed is inversely proportional to WHC (Grau and Hamm 1953).

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

Statistical Package for the Social Sciences version 9.0 (Chicago, IL) was used for the statistical evaluation of the results. Mean values and standard deviations were calculated for the resistance and the reactance measurements of fish samples in unfrozen state, after freezing-thawing and double freezing-thawing for both of the freezing rates. Normality of data was tested and confirmed using the Kolmogorov–Smirnov test.

In order to quantify the damage of tissue as a consequence of freezing, the results of the electrical measurements were presented by indices (ratios) between the reference measurements (unfrozen, fresh state, “u”) and the measurements after freezing/thawing (“f”), and between the measurements after double freezing/thawing (“fII”) and freezing-thawing according to equation 1 and 2. The results presented in this way showed the percentage of tissue damage after each cycle of freezing at a given frequency. Indices were calculated separately for fast and slow freezing.

\[
R: \% = \frac{(R_u - R_f)}{R_u} * 100 \quad \text{or} \quad \% = \frac{(R_f - R_{fII})}{R_f} * 100 \quad (1)
\]

\[
X: \% = \frac{(X_u - X_f)}{X_u} * 100 \quad \text{or} \quad \% = \frac{(X_f - X_{fII})}{X_f} * 100 \quad (2)
\]

To test the difference between fast and slow frozen samples (frozen or double frozen) in electrical, physical and chemical parameters, nonparametric Mann–Whitney U-test was used. When the results showed that there was no statistical difference between the groups, slow frozen and fast frozen groups were treated as one group (frozen or double frozen), and analysis of variance (ANOVA) was used to test the difference between unfrozen, frozen and double frozen samples. The level of significance was set at \( P < 0.05 \).

RESULTS

Figure 1 shows the resistance and the reactance curves of slow frozen (a) and fast frozen fillets (b) in unfrozen, frozen-thawed and double frozen-thawed state.

The resistance decreases with frequency in a lower frequency region, but becomes unaffected by frequency from 5 kHz upwards (Fig. 1). However, from 20 kHz, the resistance measured on unfrozen fish is again dependent on frequency, whereas the values measured on frozen-thawed and double frozen thawed mackerel remain constant for both of the rates of freezing.

The reactance is also dependent on frequency up to 10 kHz, whereas at high frequencies, as in the case of
resistance, the reactance curve of the unfrozen muscles is clearly separated from frozen and double frozen ones. The significance of lower and higher frequencies in detecting structural differences was previously reported for the farmed fish species (Vidaček et al. 2008a,b). Medium frequencies (10–50 kHz) have more importance in the prediction of the chemical composition of muscles (Petrak et al. 2001).

Table 1 shows the calculated indices of bioelectrical data which, presented in this way, may quantify a difference between three different states of fish muscle (unfrozen versus frozen-thawed and frozen-thawed versus double-frozen thawed state). The freezing and thawing can damage the tissue and theoretically, double freezing and thawing might damage it even more, so the aim of the calculation was to evaluate the percentage of this damage. Selected frequencies in Table 1 represent low, medium and high frequencies.

The first part of Table 1 shows that tissue damage after freezing is relatively poorly detected by resistance, with the maximal difference of 17.1% measured at 1,000 kHz between slow frozen-thawed and unfrozen fish. The double freezing appeared not to have considerable influence on tissue damage (2–6%). Our results are in accordance with the results of Yu et al. (2004) who measured the resistance during freezing and thawing at 100 Hz. 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 (indices were 3.61–13.54%), and the authors concluded that damage was mostly caused by the first freezing–thawing cycle and that additional cycles hardly affected the structure and properties of the tissue (Yu et al. 2004).

On the other hand, it is also shown in Table 1 that the differences between the freezing cycles could be better detected by measuring reactance. Differences between the freezing cycles are similar or lower than between the frozen and unfrozen samples (except for slow freezing at low frequencies), but the percentage of tissue damage after the second freezing cycle should not be completely overlooked. These results are also in agreement with the ones on farmed sea bass (Vidaček et al. 2008a).

As the curves of fast and slow frozen samples in Fig. 1 were fairly similar, Mann–Whitney U-test was used to test their differences. The results show that there is no difference in the resistance values between slow frozen and fast frozen groups. In contrast, the reactance measurements from 150 kHz upwards are significantly different when the slow and fast frozen samples are compared. The similarity in the resistance values is also confirmed when the double fast frozen and double slow frozen samples are tested; however, the difference between double frozen groups is significant in reactance measured from 50 kHz upwards.

Table 2 shows the results of testing the differences in electrical parameters among unfrozen, frozen and double frozen samples (ANOVA) for the frequencies at which there were no statistically significant differences between slow frozen and fast frozen samples. The differences in physical and chemical properties of fish muscle are also presented in Table 2. By Mann–Whitney U-test was previously confirmed that there were no differences between slow frozen and fast frozen samples in these properties.

The resistance of the samples measured at frequencies higher than 500 kHz and the reactance measured between 0.1 and 15 kHz are significantly different for different tissue conditions. However, the mean values of the electrical data for each condition in Table 2 may imply that only reactance can differentiate among all three conditions since the mean values

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>(U-S)/U 100 (%)</th>
<th>(S-F/S)/S 100 (%)</th>
<th>(U-F)/U 100 (%)</th>
<th>(FF-FII)/F 100 (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>–7.8</td>
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<td>–3.8</td>
<td>4.1</td>
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<tr>
<td>50</td>
<td>–7.5</td>
<td>–2.1</td>
<td>–5.4</td>
<td>4.0</td>
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<tr>
<td>500</td>
<td>–14.8</td>
<td>–2.5</td>
<td>–14.1</td>
<td>3.4</td>
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<td>–2.7</td>
<td>–16.7</td>
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<thead>
<tr>
<th>Frequency (kHz)</th>
<th>(U-S)/U 100 (%)</th>
<th>(S-F/S)/S 100 (%)</th>
<th>(U-F)/U 100 (%)</th>
<th>(FF-FII)/F 100 (%)</th>
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<td>–19.5</td>
<td>–21.3</td>
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<td>–24.2</td>
<td>–28.8</td>
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<tr>
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<td>47.3</td>
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<td>91.8</td>
<td>39.2</td>
<td>100.4</td>
<td>84.2</td>
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U, unfrozen samples; SF, slow frozen samples; SFII, double slow frozen samples; FF, fast frozen samples; FFII, double fast frozen samples.
of resistance for the frozen-thawed and double frozen-thawed samples are rather similar and probably statistically nonsignificant (it was impossible to test which condition differs from which). It should be emphasized though, that the results obtained at low frequencies might be influenced by parasitic impedance as a consequence of two-electrode measuring setup used. The electrical impedance of these electrodes can affect the measured impedance. These effects or artifacts can be measured as part of the system response and can be considered to be unwanted parasitic effects which alter the measurement of the actual system.

pH has the same values after both of the freezing cycles as in the unfrozen state, while there is a difference between the different tissue conditions in water content and WHC. Nevertheless, as in the case of resistance, from the mean values and standard deviations in Table 2, it could be assumed that moisture content and WHC do not differ after double freezing in respect to only one freezing-thawing cycle.

**DISCUSSION**

In fish and meat industry, it is been recommended that freezing should be fast. Generally, during fast freezing small ice crystals are formed intracellularly and extracellularly, which result in generally less damaged muscle tissue after thawing. Slow freezing has been reported to cause increased drip loss, due to diffusion of water outside the cells and subsequently, formation of larger crystals extracellularly (Kovačević 2001). During thawing, water is not incorporated inside the cells and is lost as drip. Initial advantages of using fast freezing are reported by most researchers (Pan and Yeh 1993; Chen and Pan 1997), although, in other studies of fast freezing, no advantage was observed (Cutting 1977). In our study, the difference between slow and fast frozen muscles is measured only by reactance at high frequencies.

During freezing, muscle proteins are dehydrated and denatured, and membranes are destroyed. The denaturation of fish muscle proteins during frozen storage leads to a decreased water-binding capacity, and a dry, firm and tough texture. WHC is not different for fast frozen and slow frozen fillets in this study. The reason for the same results in WHC for fast and slow frozen fish muscles could be re-crystallization that might have occurred during subsequent 2-week storage. 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 (Vidacˇek et al. 2008a). Other authors also confirmed that the fluctuation of temperature increased the rate of undesirable changes in fish muscle tissue (Bilinski et al. 1981; Leblanc et al. 1988).

Small or no differences between frozen and double frozen samples could be the consequence of the cells’ disruption in the first freezing-thawing cycle so, when the double freezing-thawing takes place, most of cells are already damaged and are not prone to further disruptions.

Overall, results show that reactance is the only variable that can distinguish fast frozen from slow frozen fish samples at higher frequencies, and that may differentiate between double and single freezing cycle at low frequencies. The latter, as already pointed out, might be influenced by electrode polarization.

Reactance has already proved to be a more precise indicator of freezing history than the resistance for farmed sea bass and rainbow trout (Vidaček et al. 2008a,b). The relationship between frequency and resistance (or reactance) seems to be identical for different fish species subjected to the same freezing conditions: the resistance and the reactance of the unfrozen samples depend on frequency in the whole frequency range (except in a narrow range at medium frequencies), while the frozen-thawed and double frozen groups are mostly independent on frequency.

Recently, the dielectric properties of soy protein solutions have been measured (Ahmed et al. 2008), and the authors
have reported that the imaginary part is more sensitive to changes in ionic mobility than the real part, which partly may explain our data. Ionic mobility is proportional to the concentration of ions and was suggested to be the main factor of alterations in the electrical properties of thawed when compared with fresh meat (Charpentier et al. 1972). Kitamura et al. (2008) pointed out that impedance measurements are able to find the material properties of organic materials, such as phase change, ion concentration, water transfer and water content. According to Bircan and Barringer (2002) water and salt are the two major ingredients which influence electrical properties. Freezing causes protein denaturation, which changes WHC of the fish muscle and causes drip loss after thawing. With drip loss, some liquid is lost, together with some ions, which may result in changed electrical attributes of thawed fish tissue. However, in our study, WHC and moisture content have shown to be possibly different only among the samples frozen with different number of freezing cycles but not for those frozen with different rates of freezing which is in contrast with the results of the electrical measurements. From the theoretical background in freezing, denaturation of proteins is most likely the factor that alters the electrical properties upon thawing. Therefore, it could be assumed that the reactivity in this work may be a more sensitive indicator of protein denaturation than WHC. It was reported previously that bioelectrical measurements, although measured at microwave frequencies, can detect protein denaturation during heating of meat (Bircan and Barringer 2002).

CONCLUSION

Thawed Atlantic chub mackerel samples previously frozen with different freezing rates have different reactance at frequencies higher than 150 kHz. Although double frozen samples might also be differentiated from the frozen ones by reactance, further studies on independent samples should be done to confirm this assumption. Wild Atlantic chub mackerel showed similar bioelectrical behavior as the previously studied farmed fish species.

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