Fish quality investigations using electrical impedance spectroscopy: preliminary results

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Abstract. The consumption of seafood has increased over the last 10 years. This article analyses impedance changes of tilapia (Oreochromis niloticus) sample measured over 36 hours by using electrical impedance spectroscopy (EIS). It was also investigated the correlations between variables in order to predictive models to degradation studies. Measurements were collected every 12 hours in order to verify any change due to deterioration. The results show that measurements in both longitudinal and transverse axes are equivalent and that the sample undergoes gradual variations in the impedance. The first set of data collected in the frequency range from 0.1 to 1,000 kHz showed that resistance varied from 310.9 (@ 0.1 kHz) to 86.8 Ω (@1 MHz) and capacitive reactance varied from -8.6 to 11.5 Ω, respectively. The fourth set of data showed a decrease of 79.3% (@0.1 kHz) in the resistance part of the impedance, whereas 98.8% in capacitive reactance at 0.1 kHz. These results might suggest that there was a nutritional loss of the sample over time. Further experiments must be done over a long period of time in order to fully understand the process. EIS might be pointed out as a potential technique for fish shelf live quality control.

1. Introduction
Seafood, especially fish, has been growing in popularity among consumers due to its high protein value for health, resulting in a market of about US$50 billions a year. Consumers and producers have preferred fresh fish products due to their convenience for processing and cooking. However, its quality control is more difficult when compared to other types of meat (e.g. beef, pork and pout) [1,2]. The maintenance of fish freshness during storage has a short life shelf, deteriorating the quality of it [1,3,4]. The deterioration is due to handling processes from net to storage and microbiological processes, such as Trimethylamine and volatile nitrogenous bases. Therefore, understanding the composition and condition are important for the quality of the fish [5].

With the advancement of technology, some methods have been developed to estimate the freshness of fish, however they are still too expensive or difficult to use [3]. The impedance spectroscopy has great potential to assess the freshness of seafood, as it allows to detect tissue changes, in addition to

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being portable, non-destructive and easy to handle [5,6,7]. Although the analysis has less accuracy for complex geometries, satisfactory measurements can be achieved in organisms with simplified geometry (majority of the mass concentrated in a single volume). Besides, the law of electrical resistivity has generality in various environments, having no restrictions on the specificity of each seafood species [7]. The impedance analyses are concentrated on the samples’ electrical properties, which in biological tissues depends on the distribution of extra and intracellular fluids. Such fluids have resistive behaviour and cell membranes are assigned to have capacitive components [8]. EIS consist of applying an alternating signal over a wide frequency range and the corresponding output is measured by two electrodes placed on the sample. Due to the technique’s sensitivity, it is important to establish the most appropriate experimental settings depending on the characteristics of the sample, the environment and the type of electrode [6].

The application of EIS must contain standardized methods regarding temperature during measurements, well defined electrode geometry, electrode depth of penetration, applied pressure on the electrodes and type of sample storage [9]. Some common sources of error in this type of analysis are: electrodes in different locations for each set of measurement; methodological nonconsistances; user inexperience; temperature drift; different time period between the sample’s death and the impedance measurement. The distance variation between the electrodes, as well as their insertion in different places (dorsal or ventral side) can also cause changes in the impedance values. This is due to the electrical volume applied to the sample and the presence of different tissue in the measure, respectively. The best location for the electrodes needs to be analyzed and defined, especially when muscle fibers (as found in fish) are not oriented isometrically [10].

As some studies already have shown (Cox and Hartman (2005) [11], Duncan et al. (2007) [12], Willis and Hobday (2008) [13], Fitzhugh et al. (2010) [14] and Hartman et al. (2011) [15]), electrical impedance spectroscopy, quickly and simply, is able to estimate the condition and body composition of in vivo, post-mortem and anesthetized fish, but its functionality in frozen fish is yet to be known [5]. The use of EIS can improve current methods of quality assurance in fresh and frozen fish, such as the quality index method (QIM) [16] and the critical and control point analysis (HACCP) [17]. The QIM method relies on quality-weighted sensory assessments by trained users, making it subjective and species-specific [5]. In the study by Cox and Heintz (2009) [18], post-mortem impedance phase angles decreased with time in adult pink salmon (Oncorhynchus gorbuscha), which can be related to post-mortem processes and then to determine when the product was frozen after the fish capturing [5]. The HACCP method, on the other hand, controls safety risks from food sources, such as pathogens, dirt, decomposition, pesticides, industrial chemicals and marine biotoxins [17]. The use of EIS can provide measures of decomposition and conditions along the process, improving HACCP [5].

Literature has shown that phase angle can be indicative of health and degradation of both living and dead fish and most commercially available bioimpedance analyzers are single frequency at 50 kHz [19]. The introduction of multiple frequencies may allow better sensitivities of degradation of seafood. Seafood is one of the most perishable proteins and increasing knowledge of quality and degradation could positively affect the global seafood industry.

The objective of this article is to make preliminary investigations of the measured impedance spectra from a fresh Tilapia sample over a period of 36 hours. It also investigates the correlations between variables in order to predictive models to allow studies about degradation. It is verified the possibility of using the EIS technique as a tool for determining the quality of a fresh seafood, such as fish. The results may be used as a basis for the development of practical, portable, inexpensive and non-destructive tools for analysis the quality of fishery products.

2. Materials and methods

2.1. Measuring system
The samples were measured using the impedance spectrum (HF2IS model by Zurich Equipment) for both parts impedance real and imaginary. One of the objectives was to compare the electric impedance spectrum measurement in different electrodes set.

Four circular stainless steel electrodes of 3.2x100 mm (diameter and length, respectively) were used. The injecting signal was a sinusoidal voltage of 1 Vp over the frequency range from 100 Hz to 1 MHz, then current was measured by the converter HF2TA from Zurich Equipment. Figure 1 shows the measuring setup, where the measuring electrodes (blue ones) were placed diagonally and equally spaced by 25 mm, whereas the injecting electrodes (red ones) spaced by 55 mm.

In order to investigate the degradation process, phase angle, resistance and reactance were initially scanned for 1) correlations between variables and 2) change in measures (max/min difference or max slope). Those variables with higher correlations and maximum change in measures were then chosen to be included in predictive models. In this part of the study, an upper and lower frequency threshold were set based on the highest correlation scores between frequency (kHz) and times (R>0.90). Those thresholds plotted to see if correlations fit graphs of slopes and differences between time 1 and time 4 for phase angle, real value (resistance) and imaginary value (reactance).

Fig.1 Measuring system diagram of the electric impedance spectrum in a fresh sample of Tilapia (Oreochromis niloticus).

2.2. Sample preparation
A fresh sample of Tilapia (Oreochromis niloticus) with 110x35x25 mm was stored in a plastic container under a paper towel. The measures were taken for 36 hours, with a 12 hours interval between them. First, the sample is withdrawn from the plastic container and put in a non-conductive surface to take the measures, then relocated in the container under a paper towel to keep the sample moisture and the position of the electrodes, as shows figure 1.

3. Results
In order to establish a more suitable and stable impedance spectra, the first set of measurements were performed in both longitudinal and transverse direction over a frequency range of 10 Hz to 10 MHz. The resistance (real part) and reactance values (imaginary part) as a function of frequency were calculated from modulus and phase of impedance. It was observed that impedance data below 100 Hz were not accurate which might be due to electrode impedance. This is why we have chosen to frequency range of 100 Hz to 1 MHz to make further measurements over time. Both transverse and longitudinal resistance had a mean value of 152 Ω (±7 Ω) at 100 Hz and 65 Ω (±2 Ω) at 1 MHz. In terms of reactance, the mean value was -2.23 Ω (±0.07 Ω) at 100 Hz and 3.22 Ω (±0.26 Ω) at 1 MHz.

Four measurements were taken over a period of 36 hours, which is one in every 12 hours. Results are presented in table 1. It can be noted that both real and imaginary part of the impedance undergo a very significant change over time for any discrete frequency within the range. The real part change
over time was approximately 79.3% at 100 Hz whereas 35% at 1 MHz. In terms of imaginary part of
the impedance, it was measured a change of approximately 98.8% at 100 Hz but 54% at 1 MHz.

Figure 2 shows the Nyquist plot for all measurements collected in the period of 36 hours. It can be
seen that the sample characteristic is visually different over time in terms of impedance changes. Each
measurement represents a semi-sphere with different radius, which is the point where the imaginary
part of the impedance is achieved. This is approximately 77, 29, 17 and 6 Ω for measurement 1, 2, 3
and 4, respectively. It worth mention that the positive values for the imaginary part of the impedance
were inverted (i.e., -ImZ) to better present the data as it is usually done for a Nyquist plot. Therefore,
the negative imaginary values might be explained by a 180 degree shift in the impedance phase, but
also may not represent any biological meaning rather than stray capacitance effects over the
measurements.

Table 1. Real and imaginary part of the impedance measured in a period of 36 hours, having the first
measurement (Meas 1) as time zero.

| Frequency (kHz) | Meas 1 (Ω) | Meas 2 (Ω) | Meas 3 (Ω) | Meas 4 (Ω) |
|-----------------|------------|------------|------------|------------|
| 0.1             | ReZ = 310.9 | ReZ = 139.8 | ReZ = 103.0 | ReZ = 64.5 |
|                 | ImZ = -8.6  | ImZ = 0.7   | ImZ = -0.7   | ImZ = -0.1   |
| 5.0             | ReZ = 281.0 | ReZ = 138.9 | ReZ = 101.7 | ReZ = 72.6 |
|                 | ImZ = -39.8 | ImZ = -9.6 | ImZ = -5.9 | ImZ = 0.4 |
| 50.0            | ReZ = 173.6 | ReZ = 109.9 | ReZ = 85.1  | ReZ = 70.4 |
|                 | ImZ = -75.2 | ImZ = -27.7 | ImZ = -16.2 | ImZ = -4.7 |
| 1,000           | ReZ = 86.8  | ReZ = 68.5  | ReZ = 58.8  | ReZ = 56.4 |
|                 | ImZ = 11.5  | ImZ = 1.1   | ImZ = 2.8   | ImZ = 5.3   |

Figure 2. Results of electrical impedance measurements during the sample deterioration process.

The lower threshold was set to 50 kHz and the higher one to 250 kHz, as shown by the red vertical
lines in figures 3 and 4. We can be observe that, between the two thresholds set by phase angle
correlations, the slopes and differences of resistance and reactance had larger differences at higher
frequencies. The correlations were slightly stronger at lower frequencies for resistance and with
similar correlations.

4. Discussions and conclusion
Lipids are stored in different parts of the body depending on the species, requiring further studies to
define a more accurate location for taking impedance measurements [20]. In this study, measurements
taken in the transverse and longitudinal direction of the sample did not show significant differences. Due to taxonomic similarities of tilapia (*Oreochromis niloticus*) with other species, the evaluation of the best location of the electrodes is probably not necessary a big issue [9].

Most of the fish degradation occurs in the period before it’s freezeed, when it is caught, handled and processed [21]. During the performed experiment, the sample impedance values were collected for 36 hours every 12 hours, which might allow the analysis of the estimated time between the fish death and freezing, as well as the condition for consumption. It can be calculated a decrease of approximately 2 Ω per hour in the real part of the impedance. However, this is only an estimation since we do not know if this relationship is linear or not. Furthermore, much more point should be measured within one hour, suggesting an interval of 15 minutes between data.

It is known that the phase angle (=resistance/capacitance) of the body tissue represents changes in intra and extracellular water distribution, which may give information about dehydration and hydration [22]. The decrease of the phase angle reflects the degradation of fish health, as well as the loss of nutritional properties and the movement of water between the intra and extracellular spaces caused by changes in cell integrity.
**Figure 3.** Correlation between impedance variables as a function of frequency. (top) Phase angle and time. (midle) Reactance and time. (bottom) Resistance and time.
Figure 4. Correlation between slope and difference as a function of frequency. (a) Phase (b) Reactance. (c) Resistance.

Further research should be carried out to investigate potential sources of error that may affect measurements, such as temperature, post-mortem time, electrode geometry and depth penetration. Recent studies by Hartman et al (2011) [15], Hafs and Hartman (2015) [23] and Stolarski et al (2014) [24] show the accommodation of temperature in calibration models. Also, the work of Hartman et al (2015) [9] indicated that both pressure and electrode depth penetration are proportional to sample size. In addition, future research may explore impedance changes in fish that have been thawed and variations as the sample moves from frozen to thawed.

It can be concluded that the multifrequency electrical impedance spectroscopy technology has been shown a feasible tool in this first part of our investigations. It might be a promise method to improve seafood quality in this type of industry, replacing the methods currently used such as commercial bioimpedance analyzers with a single frequency of 50kHz.

Acknowledgments
The authors thank the State University of Santa Catarina (UDESC), the Research Foundation of Santa Catarina (FAPESC) for the institutional and financial support, respectively, and the Seafood Analytics Inc. for helping with the data analysis.

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