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Non-invasive measurements of post-mortem changes in dielectric properties of haddock muscle – a pilot study

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Abstract

Significant changes can be measured in the electrical properties of organs and muscle during ischemia. This will undoubtedly form the basis of new diagnostic tools and tools for assessing food quality in the future. In this paper, we present measurements of the electrical properties of haddock muscle from 1 Hz to 100 kHz as a function of time after the fish was sacrificed. Clear alpha and beta dispersions were found. Most of the alpha dispersion disappeared after a few hours. The low frequency resistance of the beta dispersion increased during the first 5 h as the fish went into rigor, and then decreased as cell destruction developed.

Keywords: Muscle; Ischemia; Electrical impedance; Dispersions

1. Introduction

Electrical impedance spectroscopy is a promising tool for characterizing the physiological state of, among others, organs and muscle tissue (Gabriel, Lau & Gabriel, 1996; Ollmar, 1997; Jossinet & Lavandier, 1998; Casas et al., 1998). Several studies have been presented where changes in electrical properties of tissue are explained on the basis of physiological changes during ischemia, and hence this may form the basis of new electrical measuring methods for the monitoring of physiological processes (Erhard et al., 1993; Ishikawa et al., 1996). In this pilot study, we wanted to explore the feasibility of using modern four-electrode and lock-in technique for the detection of such physiological changes in fish muscle and furthermore to evaluate whether the changes in dielectric properties were significant enough to make electrical methods interesting, e.g., for fish freshness assessment.

The electrical properties of muscle change considerably post-mortem. The tissue typically goes through a rigor phase a few hours after having been sacrificed and the disintegration of the tissue then gradually develops. Knowledge about the electrical changes during these stages may prove useful in different areas such as medical diagnosis of organ and muscle state, fish and meat freshness assessment, basic research on physiological changes after excision and evaluation of the correlation between in vitro and in vivo properties of tissue.

Bozler and Cole (1935) measured the electrical impedance of frog sartorius muscle from 1.1 kHz to 1.1 MHz. Measurements were first done approx. 2 h after dissection. The tissue was then stimulated in order to induce contraction, and the tissue was measured again, approx. 3 h after dissection. They found a minor arc of a circle when the data were plotted in the complex impedance plane. Between the relaxed and contracted state, R_0 was found to increase by 75%, while R_∞ increased only 2%. R_0 and R_∞ denote the resistances measured at very low and very high frequency, respectively. The significant increase in R_0 was interpreted as a reduced ionic conduction through the cell membranes.

Schäfer, Schlegel, Kirlum, Gersing and Gebhard (1998) measured on skeletal muscle of rabbits and dogs from 100 Hz to 10 MHz and found a beta dispersion whose characteristic frequency in the impedance plane, typically moved from 20 to 10 kHz and then back to 20 kHz during ischemia. They furthermore found the low frequency resistance to increase during the first 300 min

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of ischemia, subsequently decreasing up to 850 min and then increasing again. The initial increase in resistance is explained as being due to increasing edema because of osmotically induced water shifts, which reduce the extracellular volume. This effect continues also after 300 min, but is surpassed by a reduced membrane resistance caused by the opening of ion channels, leading to a net reduction of the low frequency resistance. In their model, the membrane resistance reaches a constant level after about 850 min while the extracellular resistance continues to increase, resulting in a net increase of resistance after 850 min.

In this paper, we report measurements on muscle from haddock in the frequency range 1 Hz to 100 kHz. Like Bozler and Cole and Schäfer et al., we found a significant increase in R_0 as the muscle goes into rigor the first hours after the fish was sacrificed. R_0 reaches a maximum value after about 5 h and then starts to decrease. During this time course, the measured alpha dispersion also gradually disappears.

2. Materials and methods

Live haddock was kept in water of about 5°C. The fish was sacrificed and then brought to room temperature of 23°C where it was wrapped in plastic foil after the electrodes had been applied, in order to avoid dehydration. The measurements were conducted longitudinally along the side of the fish, using "Medtronic FASTRACE 4" hydrogel electrodes applied on the fish skin in a constant current, four electrode set-up (Martinsen, Grimnes & Karlsen, 1998). The 11×23.3 mm voltage reading electrodes were separated by 8.4 mm. A Stanford Research 850 digital lock-in amplifier was used to supply the reference voltage in a transconductance circuit providing the constant current, and furthermore to measure the differential voltage and phase angle (Fig. 1). A constant current of 270 μA r.m.s. was applied, giving a maximum current density of approx. 20 μA/cm^2 in the measured volume. The muscle was measured in the frequency range from 1 Hz to 100 kHz in a 1, 2, 4, 7 sequence, and the measurements were repeated 10 times during the first 13 h post-mortem. The haddock was kept at 23°C between the measurements. Temperature measurements inside one fish showed that measurements prior to 130 min had to be rejected because the fish had not yet reached a stable temperature.

Similar measurements were also done with stainless steel electrodes inserted into the muscle. The results from these measurements were almost identical to the surface electrode results and are hence left out in this paper. This finding is most interesting, however, since non-invasive techniques are always preferable in biological and medical engineering. Hence, this is an important topic for further research. Since only a small number of fish were measured in this pilot study, no statistical data can be derived from the results.

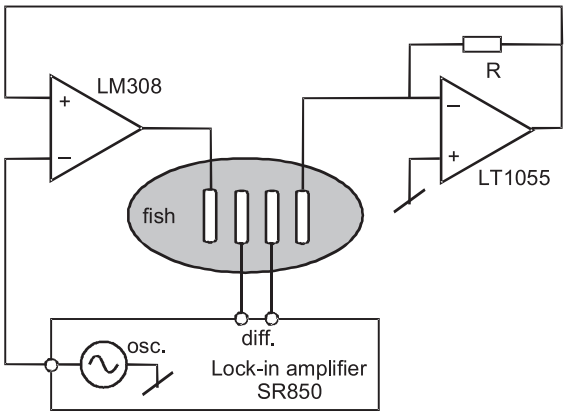

Fig. 1. Experimental set-up.

3. Results

The results from measurements on one haddock are presented graphically in Figs. 2 and 3, as Cole plots in the impedance plane (Cole, 1940). Fig. 2 shows measurements at 130, 195, 245 and 305 min after the fish was sacrificed. The 305 min measurements are also shown in Fig. 3, where also measurements at 425, 490, 550, 610 and 790 min are presented. The first measurement in Fig. 1 reveals a clear alpha and beta dispersion. As R_0 increases when the muscle goes into rigor, the alpha dispersion gradually becomes less significant. When R_0 decreases in Fig. 2, i.e. when the muscle goes from rigor into relaxation and eventually cell destruction, the alpha dispersion disappears almost entirely and only a small "tail" diverges from the beta dispersion at low frequencies. From Fig. 2, the largest changes in the electrical properties of the muscle seem to take place after about 3 h.

4. Discussion

The increased low frequency resistance in the rigor phase is most probably due to increased extracellular resistance because of cell oedema, as suggested by Schäfer et al. (1998). Resistance increase caused by reduced cell membrane conductivity, as proposed by Bozler and Cole (1935) are presumably more predominant in muscle tissue with gap junctions, as pointed out by Gersing (1994).

Bozler and Cole and Schäfer et al. did not measure the alpha dispersion of the muscle however, as their

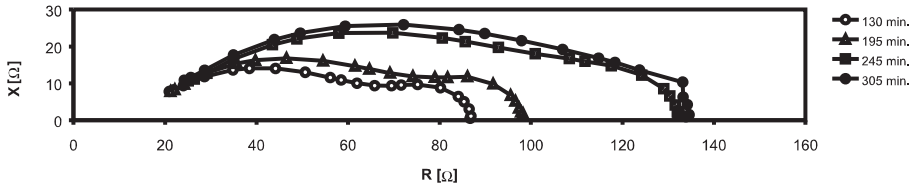

Fig. 2. Results from measurements on haddock 130–305 min post mortem.

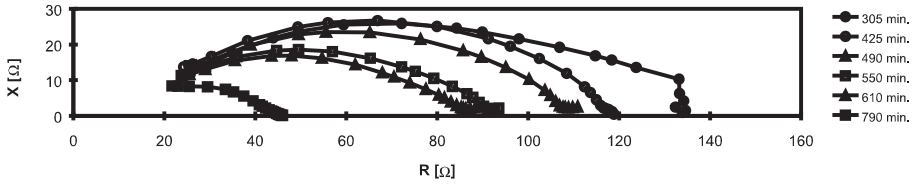

Fig. 3. Results from measurements on haddock 305–790 min post mortem.

lowest measuring frequency was 1.1 kHz and 100 Hz, respectively. Schwan (1957) suggested that the cause of the alpha dispersion in muscle is mainly counterion polarization mechanisms at the cell surface. Fatt (1964) on the other hand, argued that the alpha dispersion arises from polarization of the entrance of channel systems in the muscle fibers. Foster and Schwan (1989) concluded that both of these mechanisms probably contribute to the low frequency dispersion of muscle.

The beta dispersion arises mainly from capacitive charging of cell membranes and is described by the Maxwell-Wagner theory for interfacial phenomena between materials with different electrical properties (Foster & Schwan, 1989).

As shown by Figs. 2 and 3, the alpha dispersion is affected at an earlier stage compared to the beta dispersion. This could indicate that counterion polarization and polarization of the channel system of the muscle are more sensitive to muscle contraction and incipient cell destruction than the Maxwell–Wagner effect. However, curve fitting showed that while the characteristic frequency in the impedance plane dropped from about 60 kHz at 130 min to 26 kHz at 305 min for the beta dispersion, the corresponding change for the alpha dispersion was an increase from about 400 to 600 Hz. This reduced separation of the two dispersions may also enhance the impression of a disappearing alpha dispersion (Martinsen, Grimnes & Nilsen, 1997).

The results clearly show that fish muscle exhibits significant changes in dielectric properties post-mortem and a further study to establish the most relevant parameters to use e.g., for fish freshness assessment, should therefore be carried out.

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