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Pulsed Electric Stimulation as a Method for Assessing the Quality of Biological Objects

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Abstract—By using new computer measurement system that allows you to create conditions for reversible depolarization of cell membranes, we investigated the correlation instantly values active and capacitive components with quality indicators of muscle tissue. Electrical test of objects is represented as a large array of data. Found that individual features of the muscle tissue, depending on the species, are impact on the array. When subsequently his compared with a unique array of references, it becomes possible to express and reliable assessment of the type of muscle tissue.

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INTRODUCTION

Biological tissues possess ionic conductivity and can be qualified as conductors of the second kind. Cell membranes enclosing the cytoplasm and intracellular structures exhibit dielectric properties; they provide for sufficiently high capacitance of tissue and its polarizability [1].

Cell injury or death involves muscle protein breakdown by proteolytic enzymes, increasing of level intracellular Na^+ concentration and movement of K^+ ions across the cell membrane, whereby the biological tissue gradually loses polarizability [1]. Therefore, the capacitive component of the electrical impedance of a biological object such as, e.g., flesh of an animal immediately after slaughter takes a maximum value and then decreases in storage; the active component decreases in simultaneously. This feature enables the use of electrical characteristics of biological objects for determining their quality indicators.

The method for assessing the quality of a biological object, proposed by us, consists in the following. The object is exposed to a pulsed electric current for a certain period, and the current and voltage are measured. The resulting array of experimental data is compared with that for biological objects having known quality indicators, so the quality of the object examined (food product) can be assessed.

Electroanalysis in pulsed mode allows revealing the initiated destruction of biological membranes, caused by their forced depolarization. This is a more informative method for analysis of biological objects than that based on the use of alternating current, and it provides sufficient accuracy of measurements.

Breakdown of the lipid membrane component at selected pulsed voltage parameters leads to a sharp, though short-term, increase in cell permeability and to enhancement of ion fluxes, thereby leading to reversible depolarization of cell membranes. Moreover, this electric stimulation procedure makes it possible to eliminate the “concealing” effect, caused by tissue fluids, which prevents the appearance of capacitive resistance. Specifically the capacitive component of current and its dynamics provide reliable information

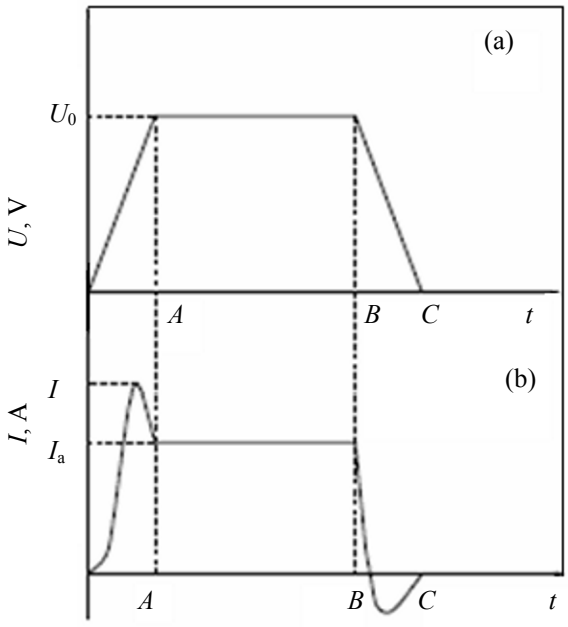

Fig. 1. (a) Trapezoidal voltage pulse formed and (b) its corresponding current pulse.

about the morphofunctional parameters of biological tissue and also allow estimating the extent of destructtion of cell membranes.

Being able of charge accumulation, cell membranes of biological tissues can be charged and recharged when electric current is passed across them, and these processes affect the electric current value. When polarization of the cell membranes changes, the rates of their charging and recharging differ depending on the degree of integrity. These differences are associated with the cell size, cell membrane structure, composition of intra- and extracellular environments and, hence, with the electrical conductivity. This leads to differences in charge localization in membranes and in the ratio of the charges accumulated.

Here, we experimentally confirmed the possibility of calculating the total pulsed current, the capacitive and active current components, specific active resistance, specific capacitance, and other parameters that serve as criteria for identifying biological objects and determining their quality indicators [2].

The value of active current, which is determined by the electrochemical processes as associated with irreversible charge transfer, is calculated by the formula:

$$I_a = \frac{SU_{pol}}{R_a}.$$

The capacitive current component is calculated as follows:

$$I_c = SC_{DC}\frac{dU_{pol}}{dt}.$$

Here I_a, capacitive current, A; S, area, m^2; U_{pol}, polarization voltage, V; C_{DC}, capacitance, F; and t, time, s.

It should be noted that the capacitance is actually pseudocapacitance because of inertia of charge motion under high-voltage field application.

The total current is the algebraic sum of the active and capacitive currents:

$$I_{tot} = I_a + I_c = S\left\{\frac{U_{pol}}{R_a} + C\frac{dU_{pol}}{dt}\right\}.$$

Here I_{tot}, total current, AI_a, active current, A; I_c, capacitive current, A; S, area, m^2, U_{pol}, polarization voltage, V; R_a, active resistance, Ω; C, capacitance, F; t, time, s.

Therefore, C and R_a are preferably determined with the use of trapezoidal polarization voltage (Fig. 1):

$$U_{tot} = U_{AO}\exp\left[-\left(\frac{t-t_0}{t_1}\right)^n\right].$$

Here U_{AO} is the polarization voltage amplitude, V; t_1, pulse length, μs; $t_0 = 0.4t_1$; and n, an even number.

Specifically the trapezoidal pulse shape allows the power supplied to be used to the fullest extent, and a prolonged interpulse pause enables the system returning to the original state. As a result, catastrophic heating of biological tissue is avoided.

With trapezoidal driving voltage used for polarization of the objects examined it is possible to directly measure the current density at a given rate of electric potential rise.

EXPERIMENTAL

To verify the theoretical model considered here, we carried out experiments on high-voltage pulsed electric stimulation at voltages up to 1000 V and voltage rise and fall rates of 10^7–10^9 V s^{-1}. In the case of a pulse group, the pulses were fed at a frequency of 0.8–2.0 Hz. By varying the voltage rise and fall rates within the limits indicated it is possible to optimize the mode of recording the electrical parameters. The minimal frequency is 0.5 Hz.

Lower frequencies of feeding pulses lead to more prolonged measurements and complicated recording of the electric signal by a digital-to-analog converter. Pulse frequencies above 10 Hz can lead to catastrophic heating of biological tissue. The interpulse pause in the pulse group should be sufficient to relaxation of biological tissue (to return to its state before electric stimulation was initiated). The preferred duration of trapezoidal pulse is 5–300 μs.

By exposing biological objects to pulses at voltage with high-speed potential's sweep, starting from 10^4 V s^{-1} it is possible to apply an increasing voltage difference to a biological material in which the cell membrane polarization process is already initiated. This allows setting the voltage required for their forced depolarization and the corresponding depolarization current.

Results obtained at higher rise rates of potential's sweep of pulsed voltage characterize the polarization of cell membranes in the initial stage of electric stimulation, as manifested in the pattern of electric current oscillations corres-ponding to the ascending part of voltage. The upper rate of voltage rise as limited by the parameters of existing electronic switches is ~10^{11} V s^{-1}.

Our study was concerned with chicken flesh (muscle tissue) taken 1, 2, 3, and 4 days after slaughtering.

The electric stimulation mode was as follows: trapezoidal-shaped DC voltage pulses, time of voltage rise to setpoint value 200 ns (voltage rise rate 10^9 V s^{-1}), interelectrode potential difference 200 V, single pulse duration 150 μs, pulse frequency 1.4 Hz, and number of pulses that passed through the electrochemical system 16.

For implementation of the electroanalytical method proposed, a device was developed on the basis of the existing equipment for measurement and control of electrical parameters of high-current pulsed processes in electrolyte solutions [3]. This device is comprised of a cathode, an anode, and a reference electrode placed in the space separating the conducting electrodes. This system allows the polarization voltage to be directly measured at the invasive electrode (anode/cathode) – biological material interface.

The distance between the stainless-steel electrodes was 8 mm; it remained constant in the course of all the measurements. The cathodic to anodic current densities was in ratio 1 : 1.

The current and voltage were recorded with the use of a computer-aided measurement system. The results

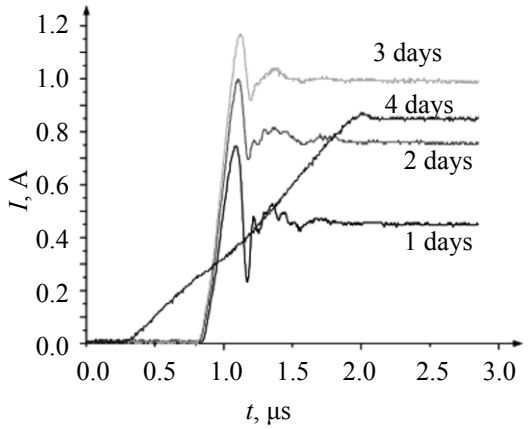

Fig. 2. Ascending parts of the chronoamperograms obtained under potentiostatic conditions (200 V) for the chicken muscle tissue samples after 1, 2, 3, and 4 days of storage.

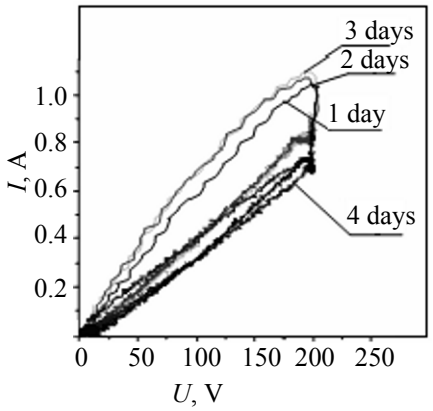

Fig. 3. Cyclic voltammograms obtained under potentiostatic conditions (200 V) for the chicken muscle tissue samples after 1, 2, 3, and 4 days of storage.

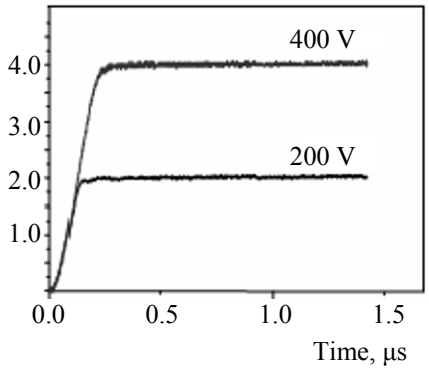

Fig. 4. Chronoamperograms obtained under potentiostatic conditions on an SMD 1206 resistor.

were digitized at specific increments and represented as a large data array. The data obtained for 16 pulses were averaged using a Gw Instek 71062A digital oscilloscope and represented graphically as the ascending parts of chronoamperograms (Fig. 2) and voltam-

Electrical parameters of the chicken muscle tissue sample

Parameters	Days of storage			
	1	2	3	4
Total current, A	1.05	1.07	1.09	0.74
Active current, A	0.72	0.80	0.83	0.70
Capacitive current, A	0.33	0.27	0.26	0.04
Proportion of capacitive current in the total current, %	31.4	25.2	23.9	5.40

mograms (Fig. 3) for different periods of storage of the object examined. From these plots, the total current and its active, reactive, and pseudo-capacitive components can be derived. The resultant data array corresponds to an individual state of bio-logical materials of both different nature and different quality.

Our experiments show that, during 1–3-day storage of the object examined, the active current tended to increase, and the ratio of the capacitive to active components of the pulsed current, to decrease. On the fourth day, the capacitive component of the pulsed current was negligible; moreover, the current rise time significantly increased. Notably, fairly dramatic changes occurred on the fourth day.

The cyclic voltammograms recorded at the voltage amplitude of 200 V for the chicken muscle tissue samples after 1, 2, 3, and 4 days of storage exhibit a steady decrease in the area enclosed by the voltammograms. This also evidences a decrease in the capacitive component of the electrical resistance of the muscle tissue.

Table summarizes the electric current data measured.

The negligible capacitive component of the pulsed current, observed after 4 days of storage, is clearly indicative of significant destruction of the chicken muscle tissue and, therefore, of its unfitness for food. This conclusion was confirmed by organoleptic data.

To avoid distortion of data from the muscle tissue examinations, we carried out analogous measurements at a known resistance in an electrolyte of a known concentration. As the electric stimulation object served an SMD 1206 precision chip resistor (100 Ω nominal resistance, 1% precision). The electric stimulation mode for the resistor and the method used for recording the electrical parameters were similar to those in the above-described experiment. As seen from the chronoamperograms in Fig. 4 that, during the period of the voltage rise to the setpoint (200, 400 V), both the capacitive component of the pulsed current and the oscillatory process are lacking.

CONCLUSIONS

Complex temporal patterns observed for the current in the analytical control method developed by us is not associated with distortion of the measurement data due to the noise introduced by measuring instruments or components of electric circuits, wires, electric contacts, electrodes, etc. Analytical signal represents the response of the measurement system, which reflects the properties of the biological object examined. The recorded responses characterize specifically the biological tissue, rather than the measurement inaccuracies caused by various factors. It was proven that the shape and dynamics of voltammograms and chronoamperograms strictly correspond to a specific type of biological tissue and its properties.

Thus, irreversible changes that occur during storage of biological tissue samples are rigorously correlated with the electrical parameters of the electrochemical processes excited by short high-voltage pulses.

The method of identifying materials of biological origin, that we described here, can be successfully applied in food industry for assessing the quality and safety of food products and raw materials used for their manufacture. Also, this method can be used in medicine for diagnosis of various diseases and estimation of the extent of pathological changes experienced by tissues and organs.

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