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# Electrical Impedance for Easily Discover Undeclared Freeze-Thaw Cycles in Slaughtered Beef Meat

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## ABSTRACT

A portable, electrical impedance spectroscopy device to monitor the bioimpedance's resistive component of beef meat by injecting a sinusoidal current of 1mA at 65 kHz was developed. In 4 slaughtered beef both right and left *longissimus dorsi* muscles where trimmed and left muscle portion was frozen to -18° C up to 7$^{th}$ day while right one was meantime maintained at 5° C. Median value of specific resistivity of not-frozen sample was about twice $\Omega.cm^{-1}$ with respect of that of frozen-thawed sample (P = 0.004). It was concluded that the device is reliable to monitoring the ripening of beef meat in situ with the possibility of reveal undeclared freeze-thaw cycles.

*Keywords*: electrical anisotropy, slaughtered bovine meat, freeze-thaw cycles, meat electrical bioimpedance, meat ripening process.

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## INTRODUCTION

From a structural point of view the trimmed meat from a beef muscle is an anisotropic tissue which is characterized by a composite network of muscle fibres bundles containing aligned myofibres surrounded from a fine endomysial envelope of connective tissue. Several structural levels that characterize the muscle's morphology have high contrasted electrical and dielectric properties. In fact, the meat's electrical properties result in part from not frequency-dependent materials which defines meat's resistance, i.e. an array of high elongated structures with high longitudinal conductance due to both intra- and extra-cellular presence of ions, which are surrounded by connective sheaths with a very low conductance. Electrical characteristics of meat also are due to frequency-dependent materials which define its capacitive reactance due to the cell membrane property to maintain the separation of the negative from positive electrical charges across it, like as an electrical capacitor. Both these electrical specificities give rise to an electric anisotropy of the muscle which is closely dependent on its histological characteristics.
In slaughtered meat, it has been found that during the post-rigor period (which begins 2 – 3 days after slaughter) the behaviour of its electrical impedance ($Z_m$) reflects major changes occurring in meat structure (Damez *et al.*, 2005), and these modifications are related to disruption of the myofibrillar organization of the cytoskeleton and of cell membranes, due to proteases activity (Kleibel *et al.*, 1983). In fact, during this time-dependent proteolysis, i.e. the meat ripening, degradation occurs in proteins with structural tears and myofibrils fragmentation together with degradation in cytoskeleton architecture (Kristensen and Purslow, 2001).These structural modifications of trimmed muscle give rise to a progressive loss of structural anisotropy from which a reduction of $Z_m$ also occurs (Lepetit *et al.*, 2002).
The optimization of the slaughtered meat maturation period, paying close attention that the maturation process takes place properly, is one of the main goals of the meat industry, particularly of the beef sector (Damez and Clerjon, 2013). In fact this production sector has to contend with the broad-ranging variability of the raw material and with the low process control on the marketed end product, especially concerning the freeze-thaw cycles that the meat slaughtered often has to suffer with a worsening of its quality. In order to guarantee good quality of meat products the industry is looking for instrumental systems to assess and certify the product quality of this food spinneret.
In a recent paper Banach *et al.* (2010) showed that only 72 hours post-slaughter a sample of bovine meat has presented its impedance module (149 Ω) which practically coincided with the module of its resistive component (145 Ω) when a current whit a frequency of 10 kHz was injected into that meat sample. From these latter data it can be argued that, when in the meat circulates an electrical current with a high frequency, the capacitive membrane-dependent component of the $Z_m$ could be totally nullified and, in the same time, the impedance module reflects only its resistive component which could consent the expression of quality attributes of meat in the form of numerical values (Banach *et al.*, 2010).
Starting from all the above considerations, the aim of this study was that of develop and test a portable and cheaper device able to assess, easy and safe, the $Z_m$ values of slaughtered beef muscles by injecting an electrical current with a high frequency which preset value surely short-circuits the capacitive reactance of the cell membranes and thereby reduces the $Z_m$ to just the intra- and extra-cellular resistive components (Battacone *et al.*, 2016).

## MATERIALS AND METHODS

### *The analog electrical model of the muscle*

Fricke and Morse (1924; 1926) firstly have described an analogous electrical model which equates biological tissue components to passive electrical elements like as resistors and capacitors connected in series and parallel.

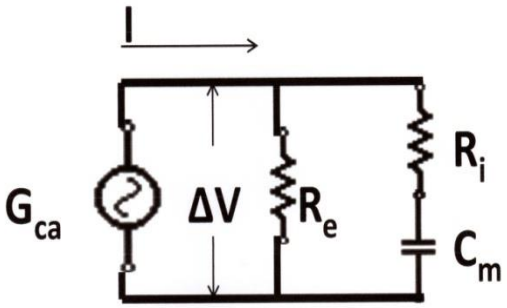


**Figure 1.** Analogous electrical model which equates biological tissue components to passive electrical elements. $R_e$ is a resistor which implements extra-cellular fluids together with some connective tissue elements and is parallel to $R_i$ which refers to resistance of intracellular fluids and is serially connected to the capacitor $C_m$ concerning electrical charges separated by the cell membranes. When the alternate current generator $G_{ca}$ is connecter to the circuit at its ends it generates a difference of electrical potential $\Delta V$ which induces the current $I$ to flow in the two parallel branches of the circuit.

So, as is shown in Fig. 1, a possible electrical model which could consent the expression of quality attributes of meat may consist of a resistor including extra-cellular fluids together with some connective tissue elements ($R_e$) connected in parallel with the resistor including intra-cellular fluids ($R_i$) which is in series with the membrane capacitors ($C_m$) (Banach *et al.*, 2010). The meat electrical impedance ($Z_m$) can be calculated as Equation (1), where $j$ is imaginary unity.

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$$Z_m = \frac{Z_s . R_e}{Z_s + R_e} = \frac{[(R_i\, R_e) - (jX_m\, R_e)]}{[R_i + R_e - jX_m]} = \frac{R_i\, R_e}{[R_i + R_e - jX_m]} - \frac{-jX_m\, R_e}{[R_i + R_e - jX_m]} \quad (1)$$

Equation (2) expresses the $X_m$ that is the capacitive reactance and *f* is the frequency of the injected current in the circuit. Equation (3) describes the $Z_s$, series impedance, formed by $R_i$ and $X_m$.

$$X_m = \frac{1}{2\pi f C_m} \quad (2)$$

$$Z_s = R_i - jX_m \quad (3)$$

It is easy to deduce that as *f* progressively increases the $X_m$ value tends towards 0, and $Z_m$ tend to the expression of Equation (4), or

$$Z_m = \frac{R_i . R_e}{R_i + R_e} \quad (4)$$

or the meat electrical impedance becomes dependent from only changes in both intra- and extra-cellular purely resistive structures which can be strongly conditioned from the ripening process of the meat slaughtered (Lepetit and Hamel, 1998).

***Instrumentation***

To reach our goal we applied a mono-frequency evaluation board with an ourself development for convert in a portable device which, on the basis of the analogous electrical model of Fricke and Morse (1924), could consent to easily assess measurements of the resistive components of the electrical impedance in the trimmed meat from beef carcasses, actuated at the production sites and with the possibility of send bioimpedance signals to a remote control center. In this light we developed the device, named $Z_{Meat}$, starting from a custom design consisting of three separate blocks: a sampling front-end, a command and control system and a data transmission system.

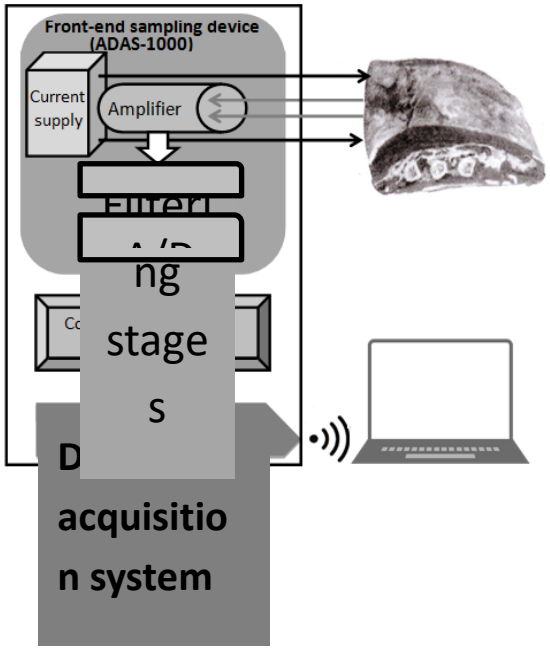


**Figure 2.** The block diagram is a schematic representation of the electrical components in the device $Z_{Meat}$.

As is shown in Fig. 2, the first block of the $Z_{Meat}$ is the sampling front-end which contains an alternate current supply that injects 1mA at 65 kHz into the meat sample by two cooper electrodes (black arrows) while other two electrodes connect the meat to the amplifier section (gray arrows). The front-end also contains a three stages filtering section to ensure the elimination of frequencies outside of the range of interest from the acquired signals. In the last stage of the front-end is made a digital conversion operation performed by a 10bit analogic-digital converter appropriately calibrated. The sampling front-end also implemented the ADAS-1000 board (Analog Devices, USA). The command and control block supervises the sampling sessions by initializing and driving the ADAS-1000's operations and sends all the information to a controlling software operating on a personal computer by the means of a data transmission system which contains a bluetooth microchip fully supporting the radio frequency communication protocol which enabled a totally wireless operation mode of the $Z_{Meat}$.

***Experimental procedures***

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Four male beef of about 16 months, half-breed belgian blue cattle with friesian, reared in the barn, were tested by the $Z_{Meat}$. The considered carcass portions were both right and left *longissimus dorsi* muscles ($LD_m$) corresponding to the VII-XI thoracic vertebrae. Trimmed $LD_m$ portions (see Fig. 3) were 25 cm length with the rostral diameter of about 15 cm and the caudal diameter of about 10 cm, and each weighing from 6 to 8 kg. Each $LD_m$ portion was collected from a local abattoir 6 hours after slaughter and was packed in a plastic bag.

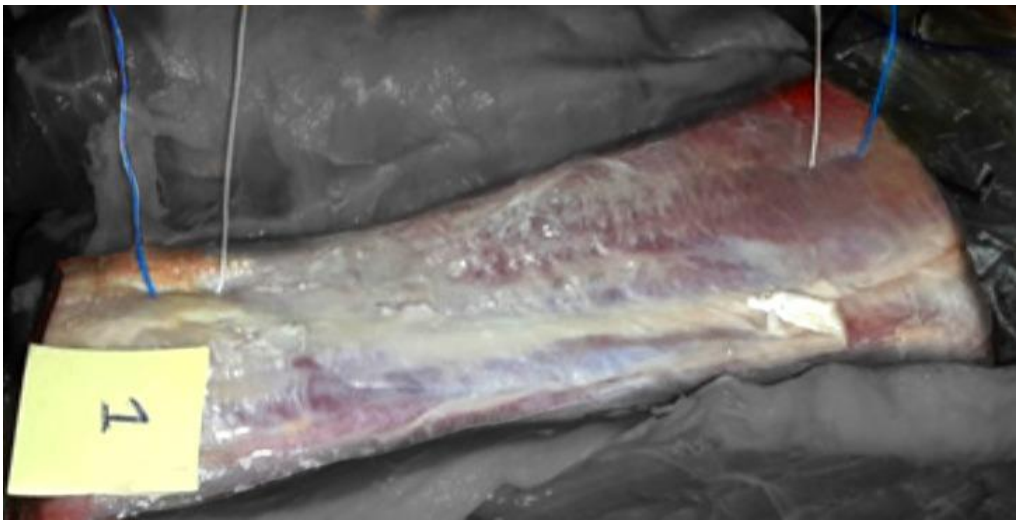


**Figure 3.** The picture shows the trimmed portion of a right longissimus dorsi muscle from one of the considered animals (285 kg heavy), which was freed from other muscular structures adjacent to it, and was oriented with its head rostral to the upper right. The two externals wires are the current injection electrodes and the two internal wires are the electrodes to detect the voltage difference during the test n°1 undertaken at the 2nd day after-slaughter.

Both $LD_m$ were immediately placed in a chilled room at 5° C. Right $LD_m$ stayed in the chilled room up to the 7th day post-slaughter while, from the 3rd until to 6th day post-slaughter the left $LD_m$ was frozen at -18° C. At the 7th day post-slaughter the left $LD_m$ was thawed in the chilled room at 5° C.

***Measurement protocol***

Experiments for $Z_m$ measurements have been undertaken into the chilling room with the following post-slaughter periodicity: 2nd day, 7th day. Fig. 3 shows that the impedance measurements were carried out by utilizing the tetrapolar scheme (Damez *et al.*, 2008) which uses two electrodes to inject a current flow $I$ into the meat sample and two different electrodes to measure the voltage $\Delta V$ between these two electrodes and to deduce electric impedance by applying the Ohm's law (Equation (5).

$$Z_m = \frac{\Delta V}{I} \tag{5}$$

The four electrodes were copper wires annealed and cold-drawn with a diameter of 0.5 mm. Each electrode was inserted manually in the sample of the meat for a 2.5 cm length. Impedance measurements were made by inserting the four electrodes on the same line, ideally located along the muscle fibres, with the detection electrodes inside those of injection. Anyway, each couple of injection or detection electrodes was placed symmetrically with respect to the ideal point corresponding to one half of the muscle length. (see fig. 3).

**Table 1.** Distances among electrodes.

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| Test n° | Inj-Det electrodes[a] Distance (cm) | Det-Det electrodes[b] Distance (cm) |
|---|---|---|
| 1 | 2.0 | 17.0 |
| 2 | 4.0 | 12.5 |
| 3 | 6.0 | 9.5 |
| 4 | 8.0 | 5.5 |
| 5 | 9.0 | 3.5 |
| 6 | 7.5 | 4.0 |
| 7 | 5.0 | 5.0 |
| 8 | 2.5 | 6.0 |
| 9 | 2.0 | 9.0 |

[a]Distance refers to each couple of injection (Inj) and detection (Det) electrodes; [b]Distance refers to the two detection electrodes

During each experimental session, from each meat sample were taken 9 measurements of $Z_m$ among which the distances within each injection-detection couple of electrodes and the distance between the two electrodes for the impedance detection, was widely varied as shown in table 1. This procedure was made to avoid possible influence on the measured $Z_m$ values depending on differences in the relative positions of the electrodes. The time span between two consecutive measurements was 10 minutes.
Sampling tests were set for a minimum duration of 20 seconds each. The $Z_{Meat}$ was configured to always operate at a sampling frequency of 2 kilosamples per second (Ksps), recording a total of about 40,000 samplings during each individual session. The standard deviation observed in every recorded sessions was equal to ± 0.01% of the average of the recorded samples (Fig. 4).

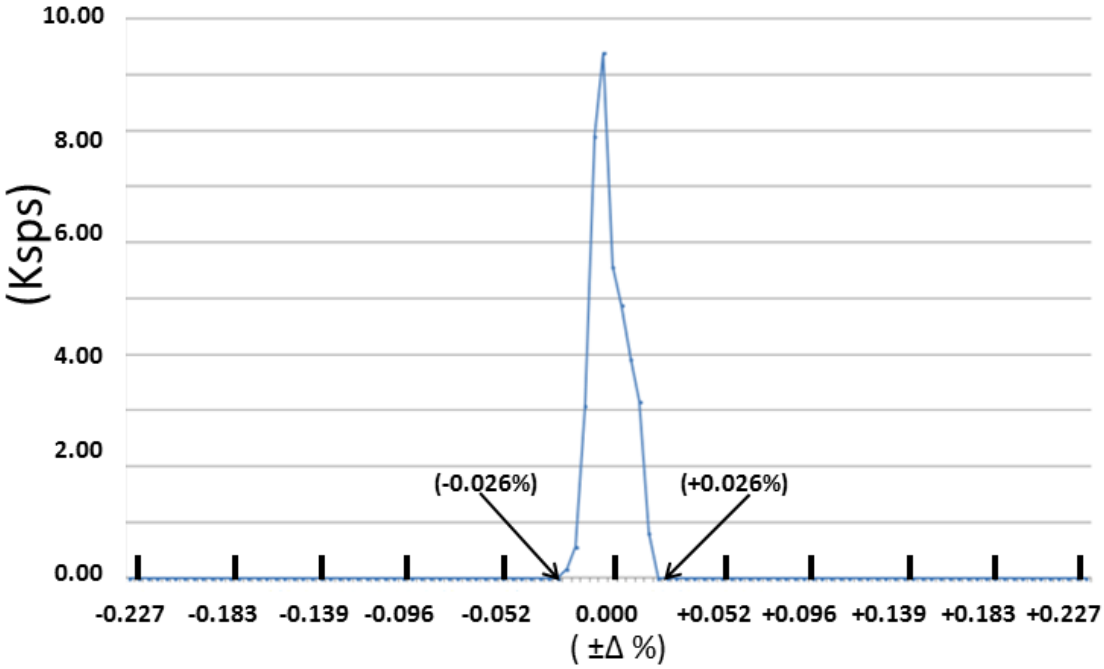


**Figure 4.** The image shows the tipical frequency distribution of about 40k samples acquired during single sampling session. In the Y axis the values are kilosamples per second (Ksps). The x axis values are reported as percentage differences(±Δ%) from the mean of the samples. As visible in the image, the entire samples population is comprised in the interval mean ± 0.026%.

***Data analysis***

Measured values of $Z_m$ were normalized dividing each of them by the distance in centimetres from the two detecting electrodes. So, specific impedance values were obtained as $\Omega.cm^{-1}$. Since these specific impedance values assessed in all the four tested carcasses were practically overlapping, so we have chosen to graphically represent the results detected by only one of the four studied animals. Due to the asymmetric distribution of the data, the visualization of the assessed values of the specific impedance was made by means of the box and whiskers plots (Krzywinski and Altman, 2014) in which we have also included the spread of data. Each box ranged from the 1st quartile (Q1) to the 3rd quartile (Q3) of the distribution, so the box length is the interquartile range (IQR), which contains the central 50% of data, and the line across the box indicates the statistical median, or Q2. The whiskers are lines extending from 1st and 3rd quartile, respectively, to the most extreme data points within Q1-1.5 x IQR and Q3 + 1.5 x IQR (Streit and

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Gehlenborg, 2014). In the chosen animal, values of $Z_m$ measured in both right and left $LD_m$ in the 2nd post-slaughter day, were put in a single data group and considered as the base data ($Z_mB$) to be compared with those measured in the 7th post-slaughter day of the right not frozen $LD_m$ ($Z_mNF$) and of the left $LD_m$ frozen ($Z_mF$) respectively. Due to the modest sample size of our data, when comparing the value of $Z_mB$ with both $Z_mNF$ and $Z_mF$ groups, we have chosen the nonparametric Wilcoxon signed rank test. In all the comparisons the statistical significance was established as a P value < 0.05. Statistical tests were carried out utilizing commercially available software (MedCalc, Belgium).

**RESULTS**

Fig. 5 shows a practically complete overlap between Q3 of the $Z_mB$ box (IQR: from 5.2 to 6.9 Ω $cm^{-1}$) and Q1 of the $Z_mNF$ box (IQR: from 4.5 to 6.4 Ω $cm^{-1}$), thus their respective median lines are very close ($Z_mB$ = 6.6 Ω $cm^{-1}$ and $Z_mNF$ = 5.8 Ω $cm^{-1}$) with no statistically significant difference between them.

The same fig. 5 clearly shows that the box of $Z_mB$ data is placed totally above the box of $Z_mF$ data (median = 3.0 Ω $cm^{-1}$ with IQR from 2.8 to 4.3 Ω $cm^{-1}$), and the median value of this latter group is lower than an half of that in the base group of data.

For this, statistical comparison between these two groups of data showed a P = 0.004.

When comparing both $Z_mNF$ and $Z_mF$ box it appears that also $Z_mNF$ box is placed above the $Z_mF$ one, and the median of no-frozen group data is 1.9 times higher than the frozen one. Also this difference among $Z_m$ values results highly significant (P = 0.004).

**DISCUSSION**

Results arising from these experimental measures of the meat electrical impedance reasonably consent to consider that 65 kHz of frequency for the applied signal for Zm monitoring, i.e. that released from the $Z_{Meat}$, as a reliable frequency to give information concerning the ageing maturation of the beef slaughtered meat.

In fact, it must be taken into account that ageing-dependent ripening of meat is due to a progressive disruption of the composite network of myofibres with their surrounding envelopes of connective tissue. This leads to an irreversible morpho-functional alteration of the cytoskeleton and cell membranes of myocytes (Kleibel *et al.*, 1983) with a progressive loss of the histological anisotrophy of the muscle tissue. This loss of the morphological anisotropy induces a parallel reduction in the muscle electrical anisotropy (Lepetit *et al.*, 2002) which is revealed by a progressive falling of its electrical impedance (Byrne *et al.*, 2000) which coincides with its real, i.e. resistive, component.

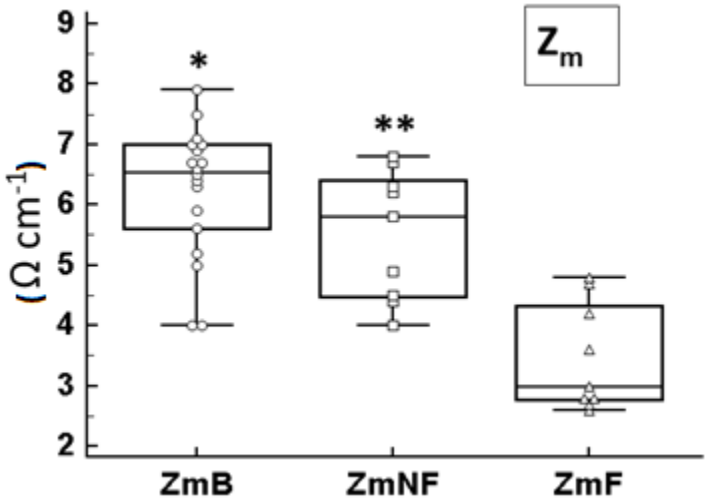


**Figure 5**. The experimental data obtained from one of the four studied animals and concerning the electrical impedance values (Zm) of the slaughtered meat in the base (FmB), not freeze (ZmNF) and freeze (ZmF) conditions, are represented as box and whiskers diagrams with markers that reveal the distribution of each measurement. With respect to ZmF: (*) P = 0.004; (**) P = 0.004.

Now considering that the $Z_{Meat}$ implements in its hardware a current generator which delivers into muscle tissue a current flow with a frequency of 65 kHz, or a frequency which is more high than 10 kHz which it has been found to nullify the muscle capacitive reactance (Banach *et al.*, 2010), then it is reasonable to assume that our $Z_{Meat}$ measures the resistive components as described in the muscle's electrical analog model (Fricke and Morse, 1924), i.e. this Zm monitor could give information on the ageing-dependent ripening of meat maturation in the form of numerical values.

Paying attention to the results from these experiments, data indicate that after 7 days from slaughter, a portion of the longissimus dorsi muscle, trimmed from a slaughtered beef and frozen at -18° C, when thawed showed a halving of its $Z_m$ with respect to the value assessed in the 2nd day post-slaughter, before its freezing. These results were in good agreement with those previously found by Banach *et al.* (2010) that, in a beef meat sample which was three months frozen at -24° C, when thawed showed a $Z_m$ which was 3.6-fold lower than that measured just after slaughter. These evidences points to remarkably better electrolytic conductance of frozen meat which is linked with damage of its structure upon the formation of ice crystals, i.e. alteration of insulating properties of cellular membranes allowing a great mobility of ions do happen (Charpenter *et al.*, 1972; Salé, 1972), whose size depends on the rate of the freezing process and the amount of water inside the structure of muscle tissue (Grujic *et al.*, 1993). In the actual experiment we also

found that, as expected, after 7 days post-slaughter the Zm of the frozen-thawed muscle sample was about a half lower than that of not-frozen one. This latter result reinforces the validity of this 65 kHz, Zm monitor to also discriminate among fresh and frozen-thawed beef meat.

## CONCLUSION

These experimental results reasonably demonstrate our applied prototype to Zm monitoring -$Z_{Meat}$- is a reliable device to assess resistive components of the electrical impedance in trimmed *longissimus dorsi* muscle of slaughtered beef since assessed impedance values by it behaved in agreement with those of previous experiments concerning the same matter.
The use of a custom designed device with a dedicated control software allowed the development of a low cost, compact, portable system device able to perform automatically and wirelessly all the measurements of the resistive components of the electrical impedance in the trimmed meat in real-time. So, this device might give some answers to the needs of the butchered meat industry especially when it is need to rapidly check, on the place of production or storage, if such beef meat is fresh or previously frozen.

## STUDY LIMITATIONS

A possible criticism for this manuscript could be moved due to the relatively small number of animals studied. However it is quite possible that calves coming from the same breeding and of the same age and weight still present very similar values of morphological and functional parameters. Nevertheless, the data reported in this paper are based on a pilot study to be followed as soon as possible by a confirmatory study based on a larger sample of tested animals.

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