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Beef meat electrical impedance spectroscopy and anisotropy sensing for non-invasive early assessment of meat ageing

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Abstract

The objective of this work was to study the electrical anisotropy behaviour of beef meat during maturation for the purpose of early assessment of meat ageing. Early assessment of beef meat fibre strength allows customised ageing of raw materials and optimisation of refrigerated storage times. During the maturation phase connection proteins break down, causing structural changes, fragmentation of myofibrils and degradation of the cytoskeleton. These modifications produce effects on the strongly anisotropic character of the muscle structure that can be observed using a sensor based on the emission of a polarised wave. For example, by tracking variations in impedance according to the angle between the electrical field direction and the main direction of fibres, a measurement of structural state, and thus of maturation state, can be obtained. In this study, two specific directions were used: along and across meat fibres. A simple method using a sensor with aligned electrodes was used to measure lineic impedances and study contact impedances as parameters of interest. A lineic impedance index was defined as the difference between lineic impedance across and along meat fibres. The lineic impedance index and the contact impedance were shown to be closely correlated to meat fibres strength. These two parameters can therefore be used to predict meat maturation state.

Keywords: Meat ageing; Tenderness; Electrical impedance spectroscopy; Anisotropy; Impedance; Sensor

1. Introduction

The use of electrical measurements to study meat goes back to the 1930s with the pioneering work of Callow (1936, 1939), who was the first to describe the basic electrical properties of meat. In the decade 1960–1970 much work was carried out in the medical field on the electric properties of biological tissues (skeletal muscles, cardiac muscle, skin, bone, etc.) (Ducrot et al., 1970; Fourcade et al., 1970; Thomasset, 1963). These studies were designed to evaluate the structural and (or) physiological integrity of these tissues. They prompted mathematical developments and the construction of models of the electrical properties of various biological tissues.

1.1. Evaluation of meat quality by electrical impedance

1.1.1. Evaluation of pH

After the first works of Callow (1936), most of the work on electrical impedance of meat published since the 1970s concerns the use of this variable to monitor fall in pH or to evaluate ultimate pH mainly in pork (Swatland, 1985) but also in beef (Byrne, Troy, & Buckley, 2000). A major quality problem in pork is preventing the production of pale soft exudative (PSE) meats, which have low pH and are strongly exudative, and so are unsuitable for processing. In beef, one quality problem is dark firm dry (DFD) meats with high pH and high susceptibility to spoiling. These two quality defects are associated with membrane modifications and changes in the extracellular medium. They are therefore bound to affect electrical properties. Most of the studies in this field have been focused on the early detection of quality defects, i.e. 45 min to 1 h after

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slaughter. However, recent results show that electrical measurements do not permit the early detection of DFD (Forrest et al., 2000; Garrido, Pedauye, Banon, & Laencina, 1994; Guerreroa et al., 2004; Jaud, Weisse, Gehlen, & Fischer, 1992). The difficulty in detecting PSE meats during the development of *rigor mortis* arises because during this time parameters such as pH and temperature are rapidly evolving and metabolic modifications sequentially affect structure and therefore electrical properties (Bendall & Swatland, 1988). Impedance (conductivity) is better able to detect PSE meats once their final pH has been reached (Guerreroa et al., 2004).

1.1.2. Evaluation of fat content

Since the 1980s many studies have been conducted on the use of electrical properties to estimate fat content in animal carcasses or muscles. Fat is a good electrical insulator and plays an important role in meat tissues impedance. Trial estimates of body composition have been made on live fish by a simple measurement of resistivity (Fauconneau, Faure, Haffray, Medale, & Vallet, 1996). Similar results have been obtained in pork by Swantek, Crenshaw, Marchello, and Lukaski (1992) and in beef and pork by Marchello, Slanger, and Carlson (1999). Slanger and Marchello (1994) measured the electrical conductivity of bovine carcasses immediately after slaughter. These measurements, associated with anatomical data, gave a fat content estimation with an excellent accuracy ($R^2 = 0.95$). This was possible because just after slaughter there is no modification of membranes or extracellular compartments, and measurements were made at a stable temperature. Madsen, Borggaard, Rasmussen, and Christensen (1999) developed an in-process instrument based on electrical impedance to measure intramuscular fat content in beef. This patented portable apparatus uses electrodes inserted in the muscle to estimate intramuscular fat in carcasses with measurements at several frequencies. Measurements of fat content made after *rigor mortis* are not consistent because the impedance in this case is also influenced by membrane modifications.

1.1.3. Evaluation of tenderness

Meat-tenderising biochemical and physicochemical processes occur during ageing. These processes include the action of endogenous proteases on the structure of muscle fibres, a progressively increase of membrane water permeability and the weakening of connective tissues.

Faure et al. (1972) set out to evaluate the state of maturation by quantifying these effects. They proposed an approach based on the ratio of impedance at low frequency to that at high frequency. This impedance ratio decreases during refrigerated storage, but Lepetit, Salé, Favier, and Dalle (2002) showed that variation in its absolute value from one animal to another could be explained by variations in ion or fat contents. Also, this impedance ratio cannot reliably indicate the state of maturation or destructuring of meat. Similar work reports on the ratio of capacity (the dielectric parameter reflecting the insulating state of the membranes) to electrical resistance (Kleibel, Pfüzner, & Krause, 1983). In this case, the measured parameters are also affected by the adiposity of tissues.

Muscle is electrically anisotropic, meaning that muscle and thus meat exhibit change in electrical properties according to the direction of the electrical fields in the sample. After *rigor mortis,* the electrical impedance of meat decreases linearly with the mechanical resistance of muscle fibres and electrical anisotropy is a better predictor of muscle fibres strength than impedance alone (Lepetit et al., 2002).

A complementary approach has been presented by Byrne et al. (2000), relating electrical properties of muscle after cooking to the tenderness assessed by Warner–Bratzler shear force (WBSF). Their results showed there was no direct relationship between meat tenderness and simple electrical measurements.

The rate of ageing in beef varies tremendously from one animal to another. The strength of muscle fibres can reach its minimum value within few days whereas for the same muscle in another animal it can take more than two weeks. It has been shown (Lepetit & Hamel, 1998) that it is possible to select meats which age rapidly if the state of ageing is known at 48 h *post-mortem*. This will avoid storing, during long period, of already aged meats. The expected benefits in storage costs are about 50%. In this study the state of ageing was measured with a destructive mechanical method but this information could be obtained from a non-destructive sensor. One such sensor using electrical impedance anisotropy was made by Damez, Clerjon, Abouelkaram, and Lepetit (2006), and has been patented (Lepetit et al., 2006).

2. Electrical properties of meat

The impedance of biological tissues and in particular of meat depends (i) on its structure, and (ii) on ionic conductivity. These two variables evolve to varying degrees as meat ages. The work of Callow (1939) showed that meat had a strong electrical anisotropy. This anisotropy was explained by the presence of long aligned muscle fibres filled with an electrolyte and surrounded by a membrane with insulating properties.

Muscle and thus meat have a strongly anisotropic structure organised in three dimensions. Muscle can be viewed as a composite network of muscle fibres surrounded by connective tissues. These fibres contain aligned myofibres. Each myofibre is enclosed in a thin endomysium sheath. These various muscle components form several structural levels that have sharply contrasting electrical and dielectric properties: the permittivity of connective tissue is very close to that measured in tendon, which is insulating (Gulino et al., 2005). Permittivity is related to the conductivity of a material (a perfect dielectric material is an insulator that is resistive to the flow of electrons). The quantity of connective tissue in meat is usually less than 2%, which means that the amounts do not affect permittivity (Foster & Schwan, 1989). The electrical properties depend on the physical and chemical parameters that determine the concentration and mobility of ions within the metabolic fluids. Electrically, meat can be simply represented by an array of highly elongated conducting cells isolated from each other by membranes. Extracellular fluids (ECF) and intracellular fluids (ICF) can be regarded as electrolytes. In muscles, Na^+ and Cl^- ions largely predominate in ECF (142 mEq/L and 105 mEq/L, respectively). In ICF, K^+ is major intracellular cation (100 mEq/L), while phosphate (PO_4^-, 142 mEq/L) and proteins (55 mEq/L) are major intracellular anions. Osmotic load is similar in intracellular medium and extracellular medium (205 mEq/L against 154 mEq/L) (Crenshaw, 1991). The charge carriers are K^+ ions, proteins and organic acids.

Two forms of cell death can occur: necrotic or apoptotic. Both can be observed in living muscle tissues, with very different morphological and biochemical consequences. Consequently to the animal slaughter, and after bleeding, deprived from nutriments and oxygen, muscle cells are conducted toward "suicide" as suggested by Herrera-Mendez, Becila, Boudjellal, and Ouali (2006). Apoptosis causes changes in quantity of free ions. Both ionic force and osmotic pressure increase between death and *rigor mortis*. It is estimated that between 60% and 80% of the increase in osmotic pressure is driven by metabolites, and the remainder by free inorganic ions not present in the cytoplasm before *rigor mortis* (Bonnet, Ouali, & Kopp, 1992; Davey & Winger, 1979; Wu & Smith, 1987). These ions, which are concentrated in organelles such as the sarcoplasmic reticulum and mitochondria, are released during membrane depolarisation after the death of the animal (Ouali et al., 2006). Although apoptosis induced changes have been extensively studied in mononucleated cells and human health, very few is known about polynucleated cells like muscle fibres. Feidt and Brun-Bellut (1996) showed that the release of Na^+, K^+, and Cl^- ions over time was not only pH-dependent but was also directly affected by cell death, in particular the rupture of membranes. In addition, Mg^{++} and Ca^{++} are linked to proteins: even when released from the sarcoplasmic reticulum after exhaustion of the ATP and inactivation of membrane pumps, these two ions can still bind to proteins with which they have a strong affinity. The final quantities of free Mg^{++} and Ca^{++} thus appear to be mainly conditioned by pH. When the pH approaches the isoelectric point (p*I*) of myofibrillar proteins (i.e. pH 5.4), the proteins charges tend to be cancelled and their capacity to adsorb cations decreases. A lower pH leads to further release of the two ions. Based on the study of Feidt and Brun-Bellut (1996), it is possible to distinguish between passive binding of the ions by proteins, which depends directly on pH, and active imprisonment, which stops as the cell's energy reserves are exhausted. The relative contributions of these two processes to ion release will vary according to the ions involved.

3. Materials and methods

We applied the most elementary and commonly used method for measuring impedance (Z) which is a complex function of alternating current frequency f, e.g.

$$Z = Z_{\text{real}} + iZ_{\text{imag}}$$

were Z_{real} is the real part (resistive), Z_{imag} the imaginary part (capacitive) and $i = (-1)^{1/2}$.

This method uses two electrodes to induce a current flow (I) in the circuit, to measure the voltage (V) between these two electrodes, and deduce electrical impedance by applying Ohm's law, $V = ZI$ (Fig. 1). In this bipolar system, electrode polarisation can produce a systematic error in the voltage measured between the two electrodes (Schwan, 1971). This is due to a polarisation zone around the electrodes where loads are created and where mobility of ions is different, and also to partial electrolysis. The result is that parasitic capacitive impedances appear at the interface of the two electrode-sample contacts and introduce a parasitic voltage drop. One way to eliminate this is to induce no current flow in the measuring circuit. This can be achieved by the quadripolar measurement method in which a current is applied by two injection electrodes and voltage is measured via two separate measurement electrodes. One other way is to use several aligned regularly spaced electrodes and to take bipolar measurements between each pair of electrodes. With homogenous samples the impedance per unit length is termed lineic impedance, so that as the distance increases the impedance also increases. Lineic impedance therefore can be calculated from the slope of the impedance plot. The contact impedance between the material and the sensing electrodes is the intercept corresponding to null distance between electrodes. The advantage from this technique is that one can obtain a multitude of impedance measures by a single application of sensing electrodes and determine contact effects.

For this study, a sensor composed of eight stainless needles was used. The needles were aligned and spaced 15 mm apart ($\varnothing = 0.6$ mm; $L = 5$ mm). Measurements were made sequentially on pairs of electrodes, allowing 28 quasi-simul-

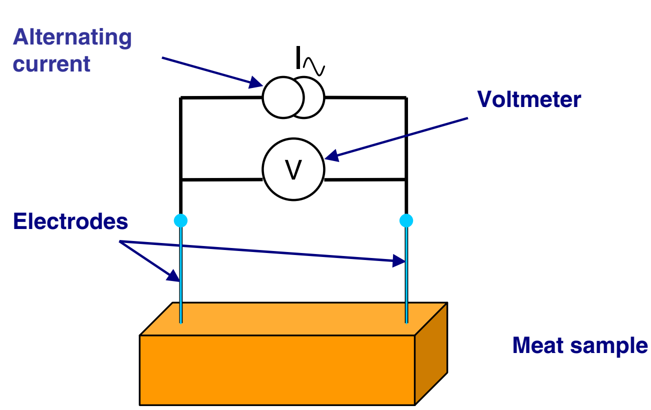

Fig. 1. Impedance measurement with the bipolar method.

taneous measures on meat samples over distances of 1.5–10.5 cm, using an impedance/gain-phase analyser (HP 4194A, Hewlett–Packard Company, San Fernando, CA) scanning 400 frequencies ranging from 100 Hz to 1.5 MHz. On each samples the sensor was applied longitudinally then transversally to the fibre direction, giving respectively lineic longitudinal impedance (Z_{lL}) and lineic transversal impedance (Z_{lT}), and allowing calculation of the lineic anisotropy index (A_{l}) where $A_{\mathrm{l}} = Z_{\mathrm{lT}} - Z_{\mathrm{lL}}$. Twenty meat samples were obtained from four *Semimembranosus* (SM), three *Rectus Abdominis* (RA), six *Semitendinosus* (SM), and seven *Pectoralis Profundus* (PP) muscles of cull cows. Muscles excised 1 h after slaughter were vacuum packed and stored for 24 h in water at 15° for a slow decrease in temperature in order to avoid cold shortening, then in a chilled room (4 °C) between measurements. Measurements were taken at 4 °C ± 1 °C from 1 to 18 days *post-mortem*.

The meat ageing was evaluated using the method described by Lepetit and Buffiere (1995). This compression method can assess ageing of meat fibres by measuring the compression stress of a meat sample at 20% deformation. Meat samples ($L = 3$; $l = 1$; $h = 1$ cm) were measured with an Instron 5543 electromechanical tester (Instron LTD Canton, MA, USA).

4. Results and discussion

The curves plotted in Fig. 2 represent variation of average impedance with distances between electrodes at 100 Hz, 10 kHz and 1000 kHz, in the transversal direction (across the meat fibres) and the longitudinal direction (along the fibres). These results pool all the beef meat samples studied: four *Semimembranosus*, three *Rectus Abdominis*, six *Semitendinosus*, and seven *Pectoralis Profundus* muscles, irrespective of their maturation state comprised between 1 and 18 days.

Fig. 2 shows that the slope of the straight line, corresponding to the lineic impedance, is both orientation-

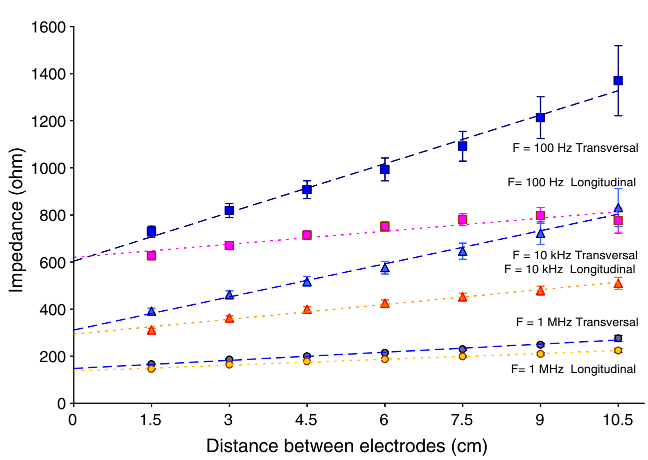

Fig. 2. Average impedance of beef meat against distance between electrodes at 100 Hz, 10 kHz and 1000 kHz in the transversal direction (across meat fibres) and the longitudinal direction (along fibres).

and frequency-dependent, while electrode contact impedances are only frequency-dependent, decreasing as frequency increases. Four points of particular interest are raised by the results shown in Fig. 2:

(i) The higher slope of impedance plots at lower frequencies is caused by the insulating effect of cell membranes.
(ii) Still at lower frequencies, dispersions in impedance values appear, caused by changes in conductivity related to ageing: non-aged meat tends to be less conductive, with higher impedance, and aged meat tends to be more conductive, with lower impedance. That could be explained by the fact that degradations in sarcoplasmic reticulum membranes during meat ageing cause a decrease in the insulating power of membranes.
(iii) Longitudinal impedances appear with lower slopes than transversal impedances underlining that measurements along meat fibres are lower than across meat fibres, current fields going along cells in extracellular fluids more than through cell membranes. Moreover, longitudinal Z slope is not frequency-dependent, unlike transversal Z slope, underlying that current flows across meat fibres go through capacitive layers (cell membranes) and that current flows along meat fibres are mainly carrier by ions in extracellular space.
(iv) Contact impedances, including impedance attributed to electrodes polarisation, which appears on plots for a null distance (to the y-intercept), are almost the same for transversal and longitudinal measurements at a given frequency, and decrease as frequency increases. This means that the contact impedance should be mainly capacitive.

Contact impedances and electrode polarisation are often regarded as parasitic phenomena or sources of error in impedance measurement, and are avoided by dedicated measurement techniques such as quadripolar sensors. In the present study, these impedances are considered as parameters of interest. Electrodes are in ohmic contact with studied samples and reflect the dielectric quality of the samples by the capacitive gap contact (Hwang, Kirkpatrick, Mason, & Garboczi, 1997). As already stated, contact impedance is almost the same in transversal and longitudinal fibre directions and this is emphasised in Fig. 3 where transversal contact impedances (Z_{cT}) are represented versus longitudinal contact impedances (Z_{cL}) at various frequencies in the range 100 Hz–1.5 MHz. These contact impedances were evaluated using the method described and correspond on Fig. 2 to the y-intercept, i.e. a null distance between electrodes. Contact impedances are frequency-dependent because they are capacitive, ranging from 600 to 100 Ω for frequencies ranging from 100 Hz to 1.5 MHz. The resistance (the real part of the impedance) could be estimated about 150 Ω from Fig. 4 at the y-intercept of the 1 MHz plot where the imaginary part of the

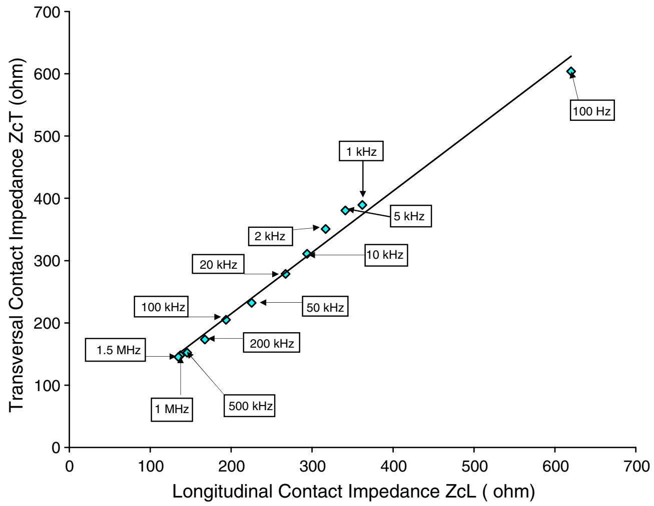

Fig. 3. Contact impedance is isotropic and increases with decreasing frequency.

Results presented in Fig. 4 are pooled from the 20 meat samples obtained from the four muscle types SB, SM, ST and PP. If each type of muscle is observed separately, correlation between contact impedance and muscle fibres strength improves markedly for certain types of muscles as shown in Fig. 5. These last results should, however, be viewed with caution given the limited number of observations per muscles.

A lineic anisotropy index (A_l) was defined as the difference between the lineic transversal impedance (Z_{lT}) and the lineic longitudinal impedance (Z_{lL}), these two last parameters being the director coefficient of the transversal and longitudinal impedance regression lines. Relationships between lineic anisotropy index (A_l) and meat fibre strength is plotted in Fig. 6 at a measuring frequency of 100 Hz. This particular frequency is in the low frequency domain where we observed the greatest difference between Z_{lT} and Z_{lL} and where major changes in electrical and

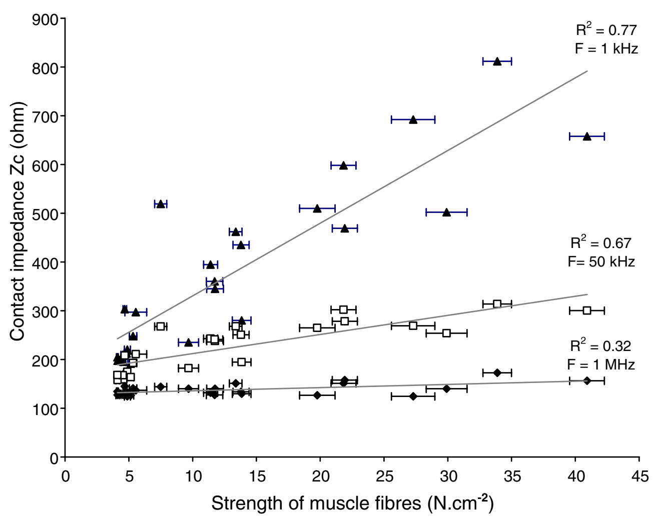

Fig. 4. Contact impedance Z_c versus strength of meat fibres for all muscle types.

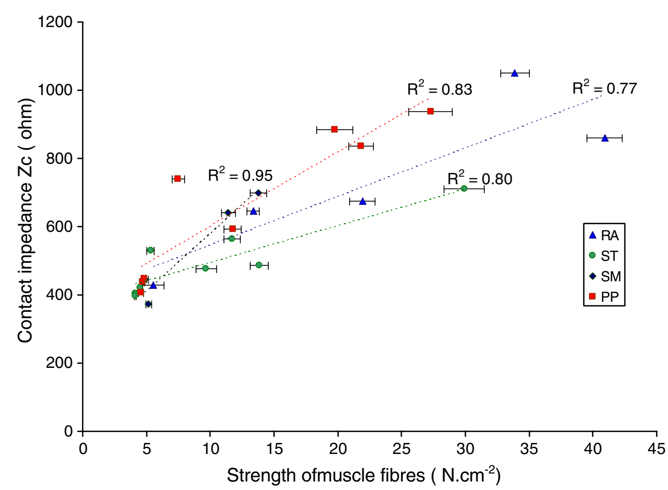

Fig. 5. Contact impedance Z_c at 100 Hz versus strength of meat fibres of *Semimembranosus* (SM), *Rectus Abdominis* (RA), *Semitendinosus* (SM), and *Pectoralis Profundus* (PP) muscles.

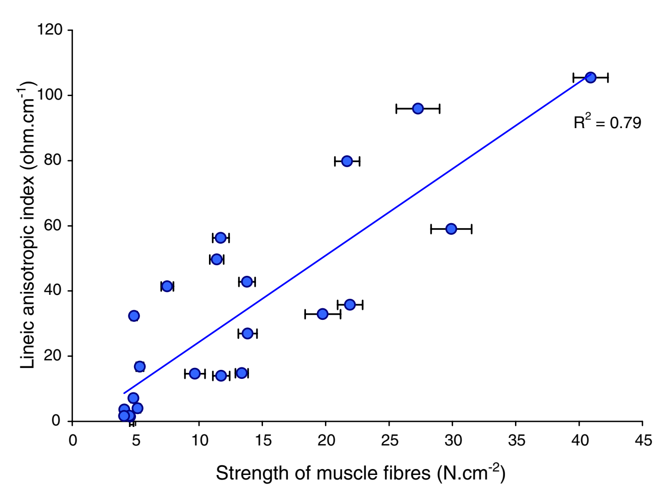

Fig. 6. Lineic anisotropic impedance index versus strength of meat fibres for all muscle types.

impedance is minimal. Contact impedances contain structural information on the meat sample and are closely correlated with the muscle fibres strength as reported in Fig. 4. On this plot contact impedances Z_c are represented as the average of Z_{cT} and Z_{cL} at given frequencies. As previously highlighted, contact impedances are predominant at low frequencies, and so the strongest linear relationship between contact impedances and muscle fibres strength appear at low frequencies.

Z_c increases with muscle fibres strength suggesting that ohmic contact impedance is higher in non-aged meat. This behaviour could be explained by drip loss occurring during meat ageing (Pliquett, Pliquett, & Robekamp, 1990) and therefore the extracellular electrolyte volume surrounding the needle electrodes which could improve contact impedance (Swatland, 1980). As these electrolytes flows out mainly from extracellular spaces (Huff-Lonergan & Lonergan, 2005), Z_c could reflect the conductive quality of ECF.

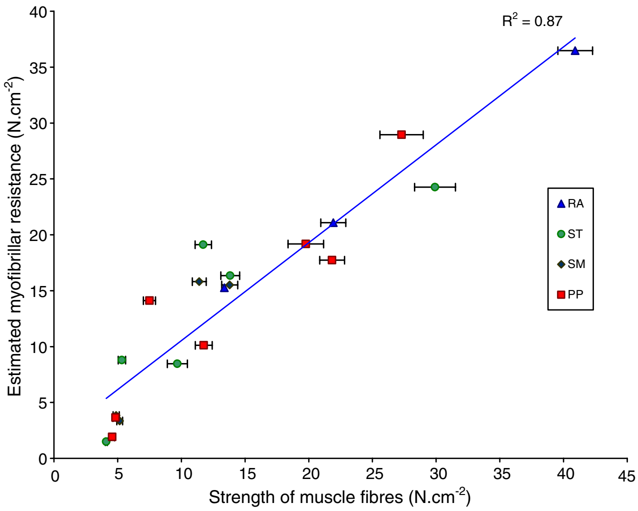

Fig. 7. Prediction of strength of meat fibres using a linear combination of the seven impedances measured respectively at 1.5, 3, 4.5, 6, 7.5, 9, 10.5 cm.

dielectric behaviour in relation with structural organisation appear in biological tissues (Damez, Clerjon, Abouelkaram, & Lepetit, 2007; Schwan, 1971). At 100 Hz a strong correlation ($R^2 = 0.79$) was observed, for the pool of all the samples studied, between lineic anisotropy index A_l and the fibre strength expressed by stress at 20% deformation. This result shows that this method using aligned electrodes can assess meat fibre strength. For better results in predicting meat fibre strength, it is possible to produce a linear combination of the seven impedances (derived from the means of individuals bipolar measurements taken, respectively, at 1.5, 3, 4.5, 6, 7.5, 9, 10.5 cm) instead of taking the director coefficient of the correlation line. Theses results are plotted in Fig. 7, and a high correlation ($R^2 = 0.87$) was obtained, showing that this improved method can be used to predict meat fibre strength.

5. Conclusion

The aim of the work presented here was to assess meat ageing states by means of a simple apparatus based on the measurement of electrical and dielectric parameters shown to be linked to meat fibre strength. Biological tissues, especially muscle, are anisotropic, i.e. their impedance varies according to whether current is propagated along or across fibres. The structural modifications of meat that occur during ageing affect not only the mechanical but also the dielectric properties of meat.

Two parameters are highlighted here; the lineic anisotropy index (A_l) defined as the difference between lineic impedance taken across and along meat fibres, and the contact impedance (Z_c) considered as a parameter of interest rather than a parasitic parameter. A_l reflects the anisotropy of meat and so reflects the behaviour of the meat structure during ageing. Z_c was shown to be isotropic in nature and reflects the conductive quality of the extracellular spaces.

Our results show that electrical measurements have high potential for use in the quality control of animal tissues. However, further research is still needed to separate the effects of the various factors such as pH, state of membranes, fat content and state of maturation.

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