NEW METHODOLOGY TO ANALIZE THE DIELECTRIC PROPERTIES IN RADIOFREQUENCY AND MICROWAVES RANGE IN CHICKEN MEATDURING POSTMORTEM TIME.

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Abstract

The biochemical and structural transformations that occur during the ageing of meat include proteolytic, electrolytic, oxidative and other processes, explain the quality and safety state of chicken meat. In this sense, the value of the dielectric properties at the relaxation frequency, in radiofrequency and microwave range have been used to monitor biochemical and structural transformations in some food processes, and can be a useful tool to predict the metabolic status of chicken meat. The aim of the work was to analyse the dielectric spectra during the post-mortem time in chicken meat, trying to understand and relate each dispersion phenomenon with the biochemical metabolism of meat ageing. 46 broiler breasts were analyzed at 5, 7, 9, 11, 13, 15, 17, 26, 50, 74, 98 and 146 h post-mortem. There were analyzed myofibrils proteins by DSC, pH, color, lactate by ion chromatography, dielectric properties in radiofrequency and microwaves and microstructure by Cryo-SEM. The proteolytic processes, main phenomenon in the ageing processes in meat, can be predicted directly through the evolution of the dielectric properties in β-dispersion, and finally, the results of this research work indicate that dielectric properties in α, β dispersions and the ionic conductivity could predict the post-mortem time in chicken meat.

Keywords: poultry meat, ageing, permittivity, radiofrequency, microwave.

1. INTRODUCTION

In the recent years, the consumption of poultry meat has had an accelerated growth and also an upward trend for the future is observed (Traffano-Schiffo et al. 2018a; Petracci et al. 2013). Particularly, the industry and consumers demand safety and high quality products. Due to this, meat ageing is one of the most influential factors that affects the final meat quality (Marino et al. 2013; Castro-Giráldez et al. 2011). Immediately after animal slaughter, complex structural and biochemical reactions are developed allowing the transformation of muscle to meat under controlled conditions (Ouali et al. 2006). These changes affect positively to the biological system, being the responsible for the tenderness, the juiciness and the flavour of the final product (Castro-Giráldez et al. 2011; Toldrá and Flores 1998). After animal slaughter, the lack of oxygen in the blood starts, thus, the aerobic glycolysis cannot continue and in order to maintain the ATP level needed to produce the vital metabolisms inside the muscle, the system follows an anaerobic pathway, obtaining lactic acid and adenosine monophosphate as final products, which mainly cause the drop of the pH (England et al. 2013) and the structural proteins degradation (Li et al. 2014). These metabolic transformations affect to the electric properties of the system.

The reduction of the pH of the muscle tissue causes the degradation of the myofibrillar structure and the increase of the liquid phase in sarcoplasmic and intercellular compartments. Intracellular and extracellular liquid phases are rich in ions with high mobility (Ca^{2+}, Cl^-, K^+ and Na^+) and also with PO_4^- (Pliquett et al. 2003; Damez et al. 2008). Protein degradation is due to the action of some endogenous enzymes, such as calpains, cathepsin and calpastatin (Chéret et al. 2007; Herrera-Mendez et al. 2006), which are the main responsible of meat tenderness. It has recently been reported that the activity of μ-calpain, m-calpain and calpastatin of chicken breasts at 48 h post-mortem decreases from 0.55±0.04 to 0.28±0.05; 2.05±0.05 to 1.99±0.01 and 1.52±0.01 to 1.19±0.03 (Units/g), respectively (Biswas et al. 2016). On the other hand, during ageing, the original size of the proteins myosin, actin and troponin-T are reduced from 250 to 56 kDa (Li et al. 2012); 43 to 32 kDa (Lametsch et al. 2002) and 70 kDa (Mudalal et al. 2014) to fragments of 28, 30, 32 and 34 kDa (Huang et al. 2011), respectively.

In this context, sensors based in the analysis of the electromagnetic field (EMF) properties in range of radiofrequency (RF) and microwaves (MW) ranges could represent a useful and non-destructive tool to analyse the evolution of the ageing especially in chicken meat, where the degradative processes occur at higher speeds in comparison to other animal species.

The EMF is a flux of photons (Baker-Jarvid and Kim, 2012) and the interaction with matter can be modeled by Schrodinger's equation (Roychoudhuri et al., 2008) attending to the quantum theory. However, at macroscopic level, it is possible to apply the Maxwell's equations (Horie et al., 2000), where the physical property that describes the electric effect is the complex permittivity and for the magnetic effect is the complex permeability (Baker-Jarvid and Kim, 2012; Pozar, 1998). Both physical properties are vectorial, defining the sense of electric and magnetic field, being possible to minimize the effect of one or other depending on the geometry of the electrodes that generate the photons flux. Focusing on the permittivity, it can be explained as a complex number, where the real term or dielectric constant (ε') is related to the electric energy storage by orientation and the imaginary term or dielectric loss factor (ε'') is related to the dissipation of the electric energy in others (Traffano-Schiffo et al. 2015; Talens et al. 2016). In RF and MW ranges, it is possible to distinguish different four effects along the electric spectra, three molecular orientation effects, α, β, and γ related with the storage and the dissipation, and one related with the molecular vibration ionic conductivity (σ), related with the dissipation. The α-dispersion, (Hz–kHz) is related with the charged molecules with high ionic strength, as electrolytes or organic acids (Kuang & Nelson 1998). The β-dispersion (kHz-MHz) is related with charged molecules, with high molecular weight and high quantity of charges (proteins, carbohydrates), and with the interfacial surfaces, with high surface tension charges (Traffano-Schiffo et al. 2017). The γ-dispersion (GHz), is related with the orientation and induction of the dipolar molecule such as water molecules (Castro-Giráldez et al. 2010a; Traffano-Schiffo et al. 2018b).

Previously, some authors the useful of the dielectric properties as online control system by many authors to determine chicken meat quality (Ghatass et al. 2008; Castro-Giráldez et al. 2010b; Damez, & Clerjon 2013; Traffano-Schiffo et al. 2018c), meat salting process (Castro-Giráldez et al. 2010c) and added water in meat (Kent, & Anderson 1996; Kent et al. 2002).

Some authors have published works on dielectric characterization of chicken in microwave range (Tanaka et al, 2000, Trabelsi, 2015; Zainal et al., 2016), or in radiofrequency (Mingjiang et al., 2011), some others in protein degradation processes in microwave (Bircanand et al., 2002), or in microwave cooking processes (Zhuang et al., 2007). Even in chicken ageing (Trabelsi, et al., 2016) where the authors worked with part of radiofrequency range and all microwave range. But few studies have analyzed the full spectrum of radiofrequence and microwaves by estimating the relaxation values of each dispersion, in order to understand complex biochemical processes and thus be able to monitor them accurately, as in chicken qualities associated with premortem stress (Traffano et al. , 2018c), to myopathies (Traffano et al., 2017; Traffano 2018a). The use of models to obtain the relaxation values in the three dispersions has been used in human tissue (Miklavcic et al., 2006), in oncology processes in human breast(Lazebnik et al., 2007) or in tissue ageing in humans (Gabriel, 2005).

The aim of the work was to analyse the dielectric spectra during the post-mortem time in chicken meat, by using Traffano-Schiffo´s model to obtain the the relaxation values of each dispersion, and try to understand and relate each dispersion phenomenon with the biochemical metabolism of meat ageing. Furthermore, the viability of using the dielectric spectroscopy as a useful tool to monitor the evolution of the meat ageing was analysed.

2. MATERIALS AND METHODS

For the experiments, 46 broiler breasts (*Pectoralis major*) from different birds, obtained from SADA Group slaughterhouse located in Rafelbunyol (Valencia, Spain), were used. After slaughter, male broilers of 42 d were bled out, plucked, tempered in a cooling tunnel at 4 ºC for 3 h post-mortem and after that, the carcasses were collected at 4 h post-mortem and carried to the laboratory of the Institute of Food Engineering for Development (IuIAD) at the Polytechnic University of Valencia (UPV) using isothermal bags with ice blocks in order to maintain the samples at 2±1 ºC. In the laboratory, the skin was removed and carcasses were deboned. The experiments were performed using chicken breasts (*Pectoralis major*) with 5 h post-mortem, maintaining them at 4 ºC during the experimental procedure.

Considering previous experiments results, the selected times to follow the ageing process of chicken meat were: 5, 7, 9, 11, 13, 15, 17, 26, 50, 74, 98 and 146 h post-mortem.

The chicken breasts used to carried out this research were classified as normal following the classification of Zhang & Barbut (Zhang & Barbut, 2005).

At the indicated times, the following determinations were made. pH was measured as was explained in Traffano-Schiffo et al. (2018c). Protein phase transitions were obtained using a differential scanning calorimeter Mettler Toledo DSC 1 (Mettler Toledo, Barcelona, Spain) provided with the full range temperature sensor FRS5 as was explained by Traffano-Schiffo et al. (2018a). Mass fraction of proteins were obtained from the transition energies and the latent heat of denaturation of the pure proteins following the method of Traffano-Schiffo et al. (2018a).

The microstructure of chicken breasts was analysed by Cryo-SEM following the method of Traffano-Schiffo et al. (2018a). A Cryostage CT-1500C unit (Oxford Instruments, Witney, UK), coupled to a Jeol JSM-5410 scanning electron microscope (Jeol, Tokyo, Japan), was used.

Lactate quantification was determined by ion chromatography (Methrom Ion Analysis, Herisau, Switzerland), using a universal standard column (Metrosep Organic Acid 250 x 7.8 mm) and a precolumn along with an eluent composed of tartaric acid (4.0 mmol/L) and dipicolinic acid (0.75 mmol/L), equipped with electronic detectors. Samples were previously homogenized at 9000 rpm in an ULTRATURRAX T25 for 10 min and centrifuged at 10000 rpm for 20 min (J.P. Selecta S.A., Medifriger-BL, Barcelona, Spain). Afterwards, 1 mL of supernatant was diluted with Milli®-Q water in a 50 mL Erlenmeyer flask. The clarified extract was filtered through a 0.45 µm Nylon Syiringe Filter (Scharlab S.L., Barcelona, Spain); 15 mL was used to analyse the lactate content. Measurements were made in triplicate.

In addition, the pH and colour of samples were measured at 12 h post-mortem in order to classify the samples as normal following the classification of Zhang & Barbut (Zhang & Barbut, 2005). Colour of the samples was measured as was explained in Traffano-Schiffo et al. (2018c).

2.1. Permittivity Measurements

Permittivity of the samples was measured in the surface of the breasts (ventral side) in radiofrequency and microwave ranges. Measurements were non-destructive.

2.1.1. Radiofrequency range

The system used to obtain the impedance consists on a non-destructive sensor as was previously described by Traffano-Schiffo et al. (2018c). The estimation of ε', ε'' from the impedance was calculated following the method Traffano-Schiffo et al. (2018c).

Finally, is possible to estimate the ionic conductivity using the loss factor, frequency and vacuum permittivity as follows:

$$\sigma = \varepsilon_0 \varepsilon'' 2\pi f \quad (5)$$

Where σ is the conductivity expressed in $S \cdot m^{-1}$.

2.1.2. Microwave range

Permittivity in the microwave range was measured with an *Agilent* 85070E open-ended coaxial probe connected to an *Agilent* E8362B Vector Network Analyser (Agilent, Santa Clara, CA, USA). The system was calibrated following the procedure of Traffano-Schiffo et al. (2018c). Permittivity measurements were measured in triplicate.

2.1.3. Modelling of permittivity data

The experimental data of the dielectric constant (ε’) were fitted by Traffano-Schiffo model (2017) (Equation 1), in order to obtain information the three dispersions that exist in RF and MW range:

$$log\varepsilon'(\omega) = log\varepsilon'_{\infty} + \sum_{n=1}^{3} \frac{\Delta log\varepsilon'_n}{1 + e^{(log\omega^2 - log\tau_n^2)\cdot\alpha_n}} \qquad (1)$$

Where n represents α, β or γ dispersion, $log\varepsilon'$ represents the decimal logarithm of the dielectric constant, $log\varepsilon'_{\infty}$ the logarithm of the dielectric constant at high frequencies, logω represents the decimal logarithm of the angular velocity (obtained from the frequency), $\Delta log\varepsilon'_n$ ($\Delta l\varepsilon'_n = \log\varepsilon'_n - \log\varepsilon'_{n-1}$) the amplitude of the n dispersion, $log\tau_n$ the logarithm of the angular velocity at relaxation time for each n dispersion, and α_n are the dispersion slopes.

2.2. Statistical analysis

The statistical analysis was carried out with the Statgraphics Centurion XVI Software (Statgraphics, Virgina, U.S.A.). One-Way ANOVA analyses were made in order to find statistically significant differences between the studied parameters. The logistic Traffano-Schiffo model (Traffano-Schiffo et al., 2017) was fitted by using nonlinear regression. Finally, the predictive algorithm was developed using the multiple regression tool.

3. RESULTS AND DISCUSSION

The biochemical transformations involved in the metabolisms of muscle transformation in meat implicate molecular changes that transform the electromagnetic equilibria in the animal tissue. By inducing a flux of photons through this tissue, it is possible to follow these metabolic transformations since they interfere in the trajectory and energy level of this flux of photons. To determine these transformations and their effect in the electromagnetic properties of photons in range of radiofrequency and microwaves, could allow the development of postmortem time prediction methodologies. In the metabolic processes that take place during the postmortem of poultry meat, changes in the content and mobility of electrolytes, phosphates or lactate can produce changes both in the α-dispersion and in the ionic conductivity (Traffano-Schiffo et al., 2018a). Changes in both myofibrilar and sarcoplasmic proteins will affect the β-dispersion (Traffano-Schiffo et al., 2018a, c), and overall structural changes will affect to the mobility of all chemical species and thus all three dispersions.

After animal slaughter, the blood flow stops, as well as the oxygen supply and, as a consequence, the aerobic glycolysis cannot continue. Muscle tries to maintain the homeostatic balance using glycogen as energy source in anaerobic conditions. Postmortem metabolism generates lactate, organic acids (Bates-Smith, 1948; Farouk & Price, 1994; Moesgaard et al., 1995) and adenylphosphates are decomposed and inorganic phosphate is produced (Moesgaard et al., 1995). The liberation of electrolytes in the plasmalemma that cause a remarkable rise in ionic strength, is caused by the mechanisms of myofibrillar proteins proteolysis because of the inability of ATP dependent calcium, sodium, and potassium pumps to function (Huff et al., 2010). During the development of rigor mortis, ion concentration in liquid phase increases: sodium, magnesium, potassium, chloride, inorganic phosphate and lactate (Feidt & Brun-Bellut, 1999). These authors found a sodium, potassium and magnesium release of 82, 66 and 22% of total amount of the muscle, respectively. During the mechanisms of apoptosis the actin-myosin interaction is weakened by the depletion of ATP and the release of Mg^{2+} and Ca^{2+}, coupled with an increase in ionic strength (Li et al., 2012). Calcium ions concentration in chicken pectoral muscle was demonstrated to increase from 70 μM after slaughter to 220 μM after 18 hours of post-mortem, remaining constant around this quantity after 5 days of meat ageing (Ji & Takahashi, 2006).

Figure 1 shows the lactate evolution during post-mortem time; the lactate content increases rapidly during the first 7 hours post-mortem. After this moment, lactate content remains constant throughout the ageing of meat.

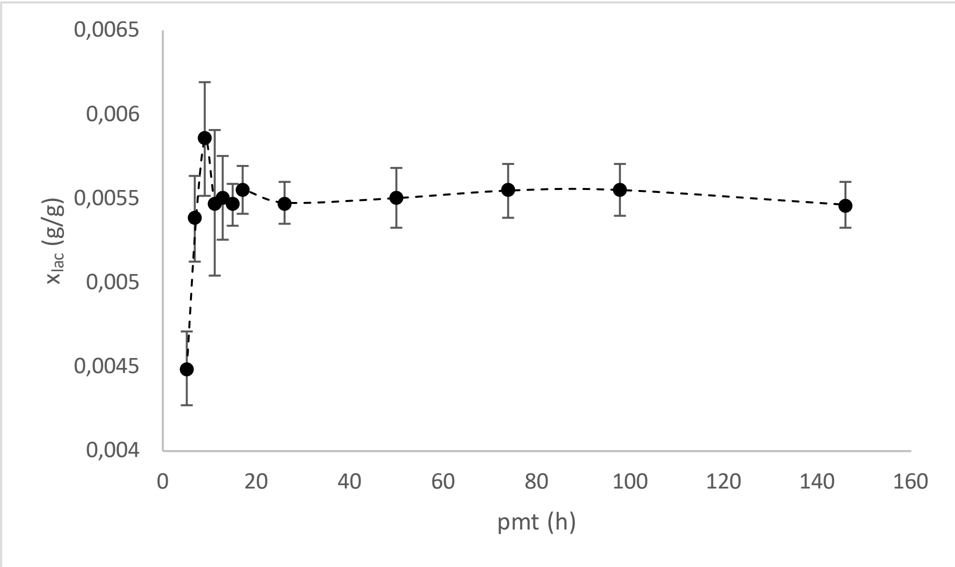

Figure 1. Lactate mass fraction evolution during post-mortem time.

The release of calcium is essential for the proteolytic processes associated with μ-calpain and m-calpain, responsible for the proteolysis of desmin and troponin (Goll et al., 1992), for protein oxidation in the proteolysis of intermediate filaments such as titin or nebulin (Huff et al, 2010), and in general for the apoptosis system that facilitates the proteolysis of the actin and myosin (Becila et al., 2010; Kemp & Parr, 2012).

In order to understand the structural changes during meat ageing, a low temperature scanning electron microscopy was performed (Figure 2). Figure 2 shows a cross section of the muscle tissue of the chicken breast, where the normal packaging of the myofibrils can be observed. Muscular fibres are involved by a thin membrane or sarcolemma and in turn, these cells are interconnected by a connective tissue or endomysium. Endomysium is the main responsible to maintain the myofibrils attached and to keep the integrity and conformation of the muscle structure. Several differences between the micrographs at 5 and 12 h of pmt as a result of the ageing process can be appreciated. At 5 h of pmt, the myofibrillar space shows a compact structure (Figure 2a, b and c); where the endomysium covers all the space between two adjacent myofibrils, without leaving space for gaps between fibres. The average thickness of the endomysium is around 3.5 μm. At 12 h of pmt, the loss of muscle integrity, the endomysium inflammation and the myofibrillar shrinkage can be appreciated (Figure 2 d, e and f). The average thickness of the endomysium at this time is around 4.8 μm, 37% higher comparing to 5 h of pmt. Also, big gaps can be appreciated.

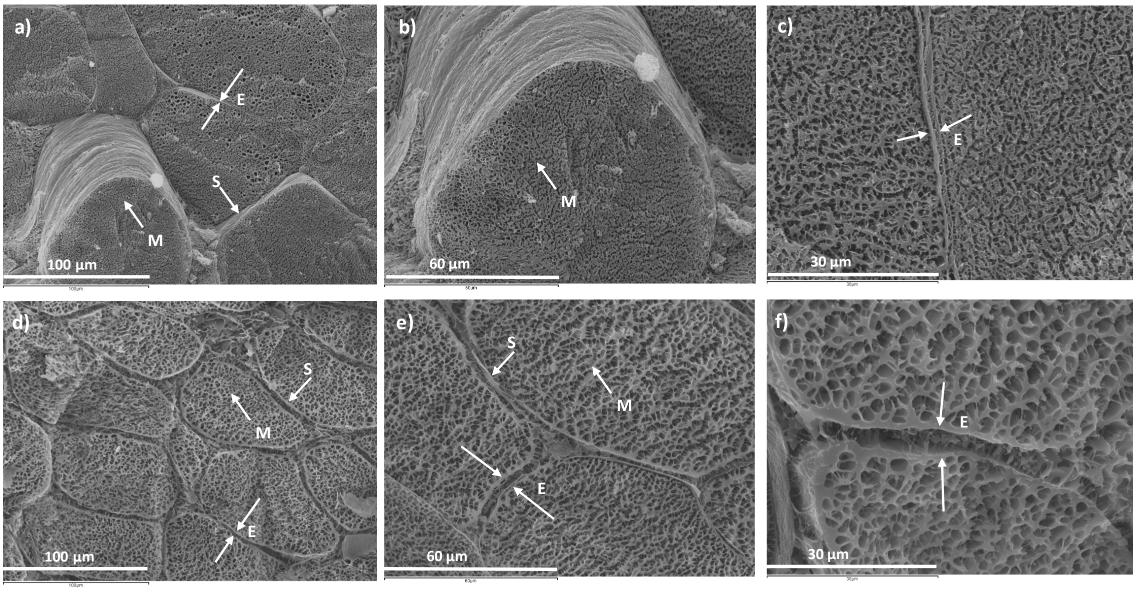

Figure 2. Micrographs of *Pectoralis major* chicken muscle. a, b and c, are samples of 5 h of pmt, d, e and f are samples of 12 h of pmt. a and d 500x, and b and e 1000x, c and f 5000x; where, E: endomysium; M: myofibrils and S: sarcolemma.

Post-mortem changes in protein degradation play a key role in the final meat quality (Hawkins et al. 2014). In order to analyse the thermal transitions of the meat proteins during the ageing evolution, DSC measurements were done. DSC curve of chicken meat shows five endothermal zones. The first peak around 55 ºC, is associated with myosin; the three central zones are related with collagen and sarcoplasmic proteins (between 63 to 76 ºC) and the last one, at 80 ºC with actin. Similar results were obtained by Fernández-Martín et al. (2000) and Ross (2006).

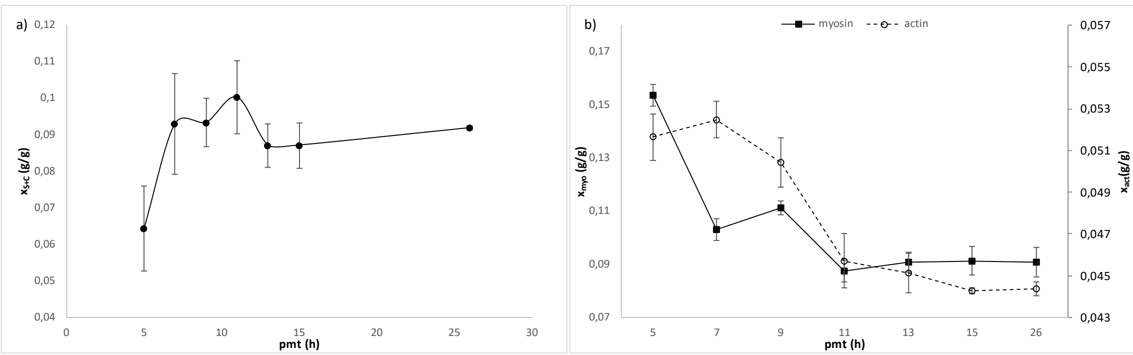

Figure 3. a) Evolution of sarcoplasmatic and collagen (—●—) proteins mass fractions during the postmortem time; b) Evolution of myosin (—■—) and actin (--○--) proteins mass fractions during the postmortem time

Rigor mortis in chicken occurs in less than 6 hours after slaughter (Alvarado & Sams, 2000), in this process the association of actin and myosin is produced irreversibely (Honikel et al., 1986). After this process, ageing of meat is produced which involves the proteolysis and results in the fragmentation of myofibrils and the lost of the integrity of muscle cells (Huff-Lonergan et al., 2010; Koohmaraie & Geesink, 2006). The proteolytic process during the postmortem is complex since it is composed by different pathways. The (μ/m) calpain, proteolitic enzime, affects the thin filaments of the myofibrils, desmin and troponin-T, weakening the strong actin-myosin interaction formed at pre-rigor facilitating its subsequent proteolysis (Li et al., 2012). Other proteolytic mechanism is given by the caspase (Boatright & Salvesen, 2003), thats affect to the degradation of specific cytoskeletal proteins including actin (Green, 2011).

Oxidative stress has also been reported to increase both calpain and caspase-mediated degradation of myofibrillar proteins, myosin heavy chain, α-actinin, actin and troponin I in myofibrils, with increasing levels of stress causing a stepwise escalation of proteolysis (Smuder et al., 2010). Figure 3a shows the evolution of sarcoplasmic and collagen content with postmortem time. It is posible to observe an increase in the content of these proteins during the 6 hours postmortem, after this time, the content remains stable with postmortem time. Figure 3b shows the decrease in actin and miosin content with postmortem time, it is posible to observe that the decrease is produced during the 11 hours of postmortem. Some authors reported the degradation of both proteins during ageing (Hwang et al., 2005; Lametsch & Bendixen, 2001; Lametsch et al., 2002; Lametsch et al., 2003)

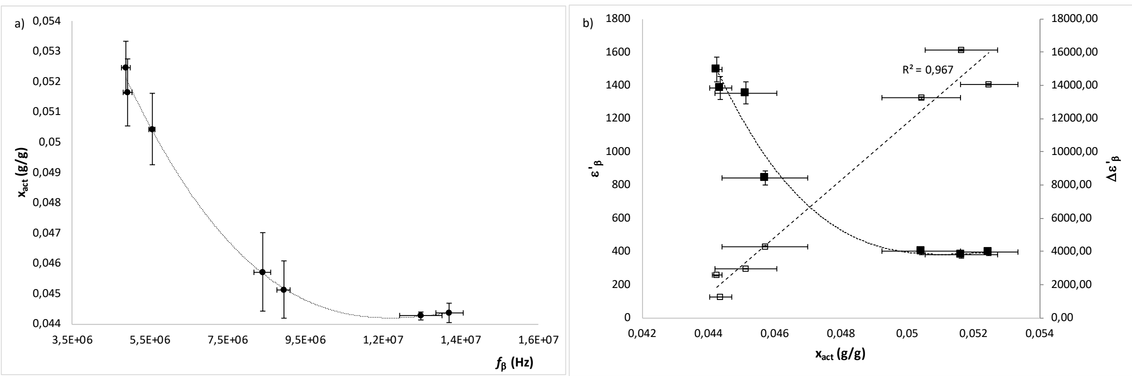

Figure 4. a) Relationship between the actin mass fraction (g/g) and the relaxation frequency in β-dispersion during the pmt. b) Relationship between actin mass fraction (g/g) and dielectric constant in β-dispersion (■) and dielectric constant amplitude of β-dispersion (□) during the pmt.

Figure 4a shows how the frequency of relaxation in the β-dispersion increases as the proportion of actin decreases, this occurs because the fragments of the actin complex that are proteolyzed are those of less molecular mass, leaving the proteins with higher molecular mass and generating a relaxation at a higher frequencies. However, these fragments are proteolyzed with a high number of active sites, they decrease the amplitude of β-dispersion, although they generate an increase in the absolute value of the dielectric constant in this dispersion, as shown in Figure 4b .

Therefore, as explained above, there are two phenomena that affect the circulation of photons through chicken tissue, the first is the release of electrolytes associated with the blockage of transport pumps, such as Ca^{2+}, Mg^{2+}, Na^{+} , K^{+}, which causes an increase in the ionic strength of the medium. This phenomenon will affect both the α-relaxation by the number of electrolytes to orientate and the conductivity of the medium, due to the increasing ionic strength. The second effect is the proteolysis of myofibrils, which generate changes in the properties associated with β-dispersion.

Figure 5 shows different dielectric properties in the dispersions α, β and γ, and the ionic conductivity with respect to the pmt. As Figure 5d shows, the ionic conductivity increases up to 80 h pmt due to the continued increase of the ionic strength associated with the release of electrolytes from the degradation of the transport pumps located in the protein tissue (Huff-Lonergan et al., 2010). This increase of the ionic strength also affects to the α-dispersion as it can be observed in the amplitude of the dielectric constant (Figure 5b). These release of electrolytes is not homogeneous since some participate in the proteolysis of the (μ/m) calpain as the Ca^{2+} and in the oxidative proteolysis as the Mg^{2+} and the Ca^{2+} (Li et al., 2012), for this reason the relaxation frequency of the α-dispersion is changing with the pmt, as figure 5a shows.

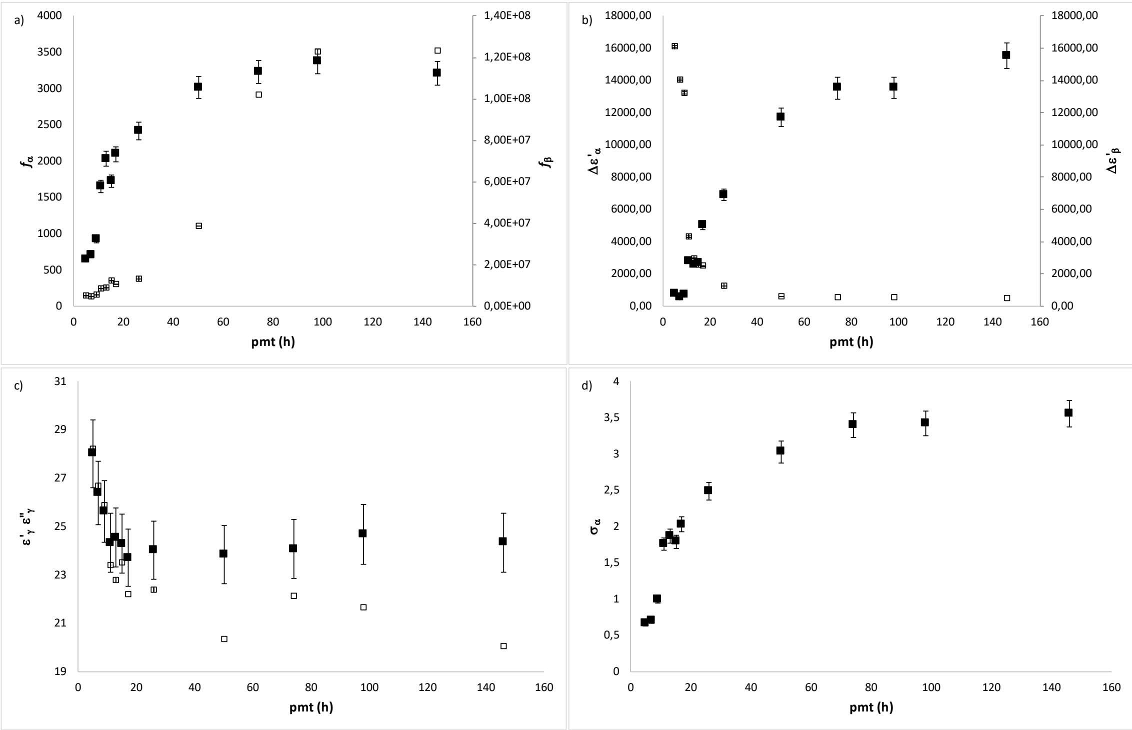

Figure 5. a) Relationship between the relaxation frequency in α-dispersion (■) and β-dispersion (□) during the pmt. b) Relationship between the dielectric constant amplitude in α-dispersion (■) and β-dispersion (□) during the pmt. c) Relationship between the dielectric constant (■) and the loss factor (□)in γ-dispersion during the pmt. d) Relationship between the ionic conductivity in α-dispersion (■)during the pmt.

The proteolysis of the myofibrillar proteins causes a decrease in the amplitude of the dielectric constant in β-dispersion until 40 h pmt, as Figure 5b shows, following the post-rigor proteolytic metabolisms described above. These proteolysis mechanisms also cause changes in the relaxation frequency of the β-dispersion with respect to proteolysis (Figure 4a) and with respect to the pmt (Figure 5a).

Moreover, the proteolysis during the meat ageing causes a reduction in the water holding capacity (Bhat et al., 2018) reducing the quantity and mobility of water molecules in the meat tissue. This phenomenon can be observed in Figure 5c, in the reduction of the dielectric constant and the loss factor in γ-dispersion during the first 20 h pmt.

Taking into account the effect of meat ageing in the α, β dispersions and the ionic conductivity, the dielectric properties can be a useful tool to predict the pmt in chicken meat.

4. CONCLUSIONS

The proteolytic processes, main phenomenon in the ageing processes in meat, can be predicted directly through the evolution of the dielectric properties in β-dispersion.

The results of this research work indicate that dielectric properties in α, β dispersions and the ionic conductivity could predict the post-mortem time in chicken meat.

5. ACKNOWLEDGEMENTS

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