

APPLICATION OF IMPEDANCE SPECTROSCOPY FOR THE CHARACTERIZATION OF HAMS DURING POST-SALTING

APLICACIÓN DE LA ESPECTROSCOPIA DE IMPEDANCIA PARA LA CARACTERIZACIÓN DE JAMONES DURANTE EL POST-SALADO

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ABSTRACT

Background: The smart control for food and feed technologies consist in the development of new methods that facilitate the control of productive processes guaranteeing the quality and safety of agrofood products. The importance given to this research line is a clear indicator of the interest of all of the agents implicated in promoting the introduction of innovative technologies. The goal is to implement new, fast, economical, analytical techniques, which constitute a real alternative to traditional control procedures. **Objective:** The aim of this study was to evaluate the use of an electronic sensor based on the impedance spectroscopy technique for the characterization of hams during the post-salting step (8, 10, 12 and 14 weeks). **Materials and methods:** 60 hams from three different categories (fat, semi-fat and lean), were tray salted under the conditions of a company ($x^{NaCl} = 0.11$ g/g dry matter). For each time, samples were first evaluated with the electronic method (non-destructive analysis) and then analytical determinations of: mass variation, moisture content, fat content, salt content and water activity were performed on each ham in different zones. The electric data were evaluated using Principal Component Analysis (PCA) and regression with Partial Least Squares (PLS) through the analytical data. **Results:** The PCA analysis discriminated between the different evaluated zones. At eighth week 79.06% of the variance was explained and at tenth week, the 82.53%. No relation was observed for the different ham categories. The correlation between the impedance signal and the analytical determinations showed the best cross validation coefficient (R^2CV) values for moisture content (0.75) and water activity (0.73). **Conclusions:** The impedance spectroscopy technique generated different signals between the zones of analysis during the first two post-salting times, eight and ten weeks, in which physicochemical differences were also observed as a consequence of the variation in the salt concentration between zones.

Keywords: dry-cured ham, quality, control, sensor, non-destructive analysis.

RESUMEN

Antecedentes: El control inteligente de los alimentos y las tecnologías alimentarias, consiste en el desarrollo de nuevos métodos que facilitan el control de los procesos productivos y garantizan la calidad y seguridad de los productos agroalimentarios. La relevancia concedida a esta línea de investigación es un claro indicador del interés de todos los agentes implicados en impulsar la introducción de tecnologías innovadoras. La meta es implementar nuevas técnicas analíticas rápidas y económicas, que constituyan una alternativa real a los procedimientos tradicionales de control. **Objetivo:** Evaluar el uso de un sensor

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electrónico basado en la técnica de la espectroscopia de impedancia para la caracterización de jamones durante el post-salado (8, 10, 12 y 14 semanas). **Materiales y métodos:** 60 jamones de tres categorías diferentes (grasa, semigrasa y magra) fueron salados en bandeja bajo las condiciones de una empresa ($x^{NaCl} = 0.11$ g/g base seca). A cada tiempo establecido, las muestras seleccionadas se evaluaron utilizando el método electrónico (análisis no destructivo) y se realizaron determinaciones analíticas de: variación de masa, contenido de humedad, contenido de grasa, contenido de sal y actividad de agua, en diferentes zonas de la muestra. La información electrónica se analizó mediante análisis de componentes principales (PCA) y correlaciones (PLS) entre la señal de impedancia y las determinaciones analíticas. **Resultados:** El análisis de PCA discriminó entre las diferentes zonas de análisis evaluadas. A la octava semana se explicó un 79.06% de la varianza de las muestras y a la décima, un 82.53%. No hubo relación entre las diferentes categorías de jamones. La correlación estadística de la señal de impedancia con las determinaciones analíticas mostró los mejores valores de coeficiente de validación cruzada (R^2CV) para el contenido de humedad (0.75) y la actividad de agua (0.73). **Conclusiones:** La técnica de espectroscopia de impedancia generó señales diferentes entre las zonas de análisis durante los dos primeros tiempos de post-salado, ocho y diez semanas, en los que también se observaron diferencias fisicoquímicas como consecuencia de la variación en la concentración salina entre zonas.

Palabras clave: jamón curado, control, calidad, sensor, análisis no destructivo.

INTRODUCTION

In the dry-cured ham process, the post-salting step consists in the penetration of ions of sodium and chloride inside the deeper areas of the ham (1). The rate of ions diffusion depends on the fat content of the ham, therefore the time of the step is variable (2). At the industrial level, the post-salting is empirically controlled by mass difference. Therefore, to quantify the salt content and diffusion in the ham it is necessary to carry out destructive determinations or the application of advanced and complex technologies, such as: computed tomography (3), magnetic resonance imaging (4, 5), magnetic resonance microscopy (6). However, impedance spectroscopy is a low cost technique that has shown good results in the evaluation of meat products (7). It bases its operation on applying an electrical stimulus to the electrodes, observing its response and determining its properties, interactions and dependencies with certain factors (8). The electrical response may vary depending on: charges present, microstructure, texture, geometry and electrolytes. Electrical impedance measurements determine or infer information about the tissue, provided that the event under analysis presents a change in dimension, in its electrical properties or conductivity (9). The experimental procedure for measuring impedance spectroscopy consists on applying a small sinusoidal electrical signal (voltage or current) to an electrode and measure its response at different frequencies resulting in an impedance value for each frequency (10). The aim of this work was the application of

impedance spectroscopy for the characterization of hams during the post-salting step.

MATERIALS AND METHODS

60 salted hams of three categories: fat, semi-fat and lean, were provided at different post-salting times (8, 10, 12 and 14 weeks) by a meat manufacturing company. The hams had a mean weight of 10.9 ± 1 kg and a pH between 5.7 and 5.9. At each sampling time electronic and physicochemical determinations were performed.

Analytical determinations

The analytical determinations were carried out on four zones obtained from a slice of approximately five centimeters of thickness extracted from the widest section of the ham as described in a previous work (11). The zones were: A, near the lean surface, B in an intermediate zone and zone C, near to subcutaneous fat (Figure 1). The R sample, represents whole of the piece, it was obtained from the grinded rest of the ham.



Figure 1. Analysed zones from the widest ham section: A, B, C.

Changes in the total mass of the ham (ΔM_t post-salting) throughout the post-salting step were estimated from the weight (M) of the ham (at sampling time t and 0) (Equation 1):

$$\Delta M_t = \left(\frac{M_0 - M_t}{M_0} \right) \quad \text{Equation 1}$$

Moisture content was determined according to the Association of Official Analytical Chemists AOAC official method (12). Fat content was carried out by extraction in the Foss-Soxtec 2055[®] equipment with petroleum ether following the AOAC official method (12). Water activity (a_w) was measured at 25°C from the grinded samples; a dew point hygrometer (DECAGON Aqualab CX-2[®], ± 0.003 , Pullman, WA, USA) was used. The salt content was quantified following the method described in Girón *et al.*, (2015) (13). All determinations were done in triplicate. The total sodium chloride concentration in the liquid phase (z^{NaCl}) was estimated from the weight fractions of water (x^w) and sodium chloride (x^{NaCl}) (Equation 2).

$$z^{NaCl} = \left(\frac{x^{NaCl}}{x^w + x^{NaCl}} \right) \quad \text{Equation 2}$$

Electronic measurement

A low-cost, flexible, light, non-destructive measurement system was developed by the Interuniversity Research Institute for Molecular Recognition and Technological Development (IDM) at the Universitat Politècnica de València (UPV) (14). This impedance spectroscopy measurement system applies an electric signal to the sample and measures the response in a frequency sweep between 1 Hz and 1MHz, generating a sinusoidal signal, with an amplitude of up to 1 Vpp. The system consists of a software application that runs on a computer and the electronic equipment, doing a frequency sweep getting modulus and phase of impedance, for each frequency 256 points are calculated which correspond to the evolution of the signal. A Fourier analysis is executed in order to calculate the amplitude and phase, from this data, the software calculate the modulus and phase for all the frequencies. Frequency values of impedance spectroscopy used by other authors were taken into account (15, 16). A double electrode was used, composed of two stainless steel needles 15 mm long and 1 mm in diameter, separated by a distance of 10 mm in a nonconductive frame previously used in other studies (17).

Statistical analysis

A multifactor ANOVA was conducted in the first part of the study for each physicochemical parameter to evaluate the effect of post-salting time, ham type, and sampling zone. The least significance difference procedure (LSD) was used to test for the differences between averages at the 5% level of significance. The data were statistically processed using Statgraphics Centurion XVI statistical package. (Manugistics Inc., Rockville, MD, USA).

Principal Component Analysis (PCA) was conducted in order to evaluate the electronic data. Partial least squares regression (PLS) was carried out in order to correlate the physicochemical information with the electronic measurements. The accuracy was given by the root mean square error for calibration (RMSEC), cross validation (RMSECV) and coefficient of determination (R^2) for calibration (C) and cross validation (CV). Selection of the main frequencies was obtained from the iPLSDA genetic algorithm of interval selection (). All multivariate analyses were performed using PLS Toolbox 6.3 (MATLAB PLS Tool-box. Eigenvector Research, Inc., Wenatchee, Washington, USA).

RESULTS

Physicochemical characterization

At the end of the post-salting step, the mass variation (ΔM_t post-salting) for each ham type showed an average value of $-0.178 \text{ kg} \pm 0.007$ fat, $-0.200 \text{ kg} \pm 0.006$ lean and $-0.205 \text{ kg} \pm 0.004$ semi-fat. Figure 2 shows the fat content of the samples at 8, 10, 12 and 14 weeks of post-salting.

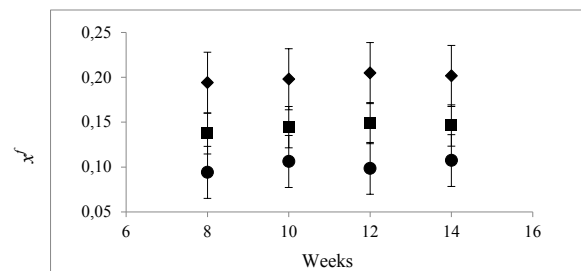


Figure 2. Mean values \pm standard deviation of fat content (x^f) for the R zone at 8, 10, 12 and 14 post-salting weeks. Ham type: fat (◆), lean (●), semi-fat (■).

Figure 3 shows moisture content (x^w , g/g), salt content (x^{NaCl} , g NaCl/g), and water activity (a_w) in A, B, C and R zones at 8, 10, 12 and 14 weeks of post-salting.

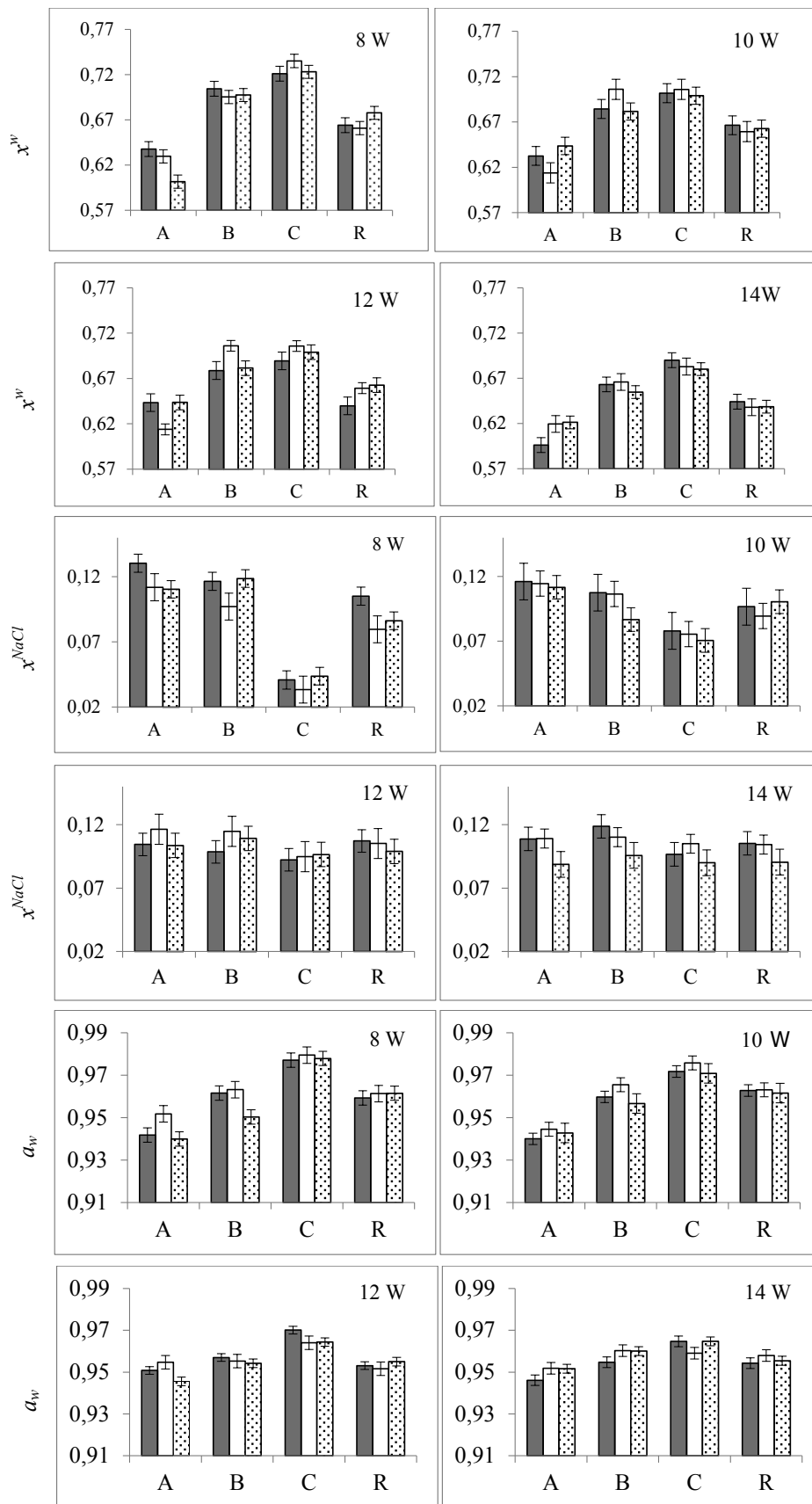


Figure 3. Mean values \pm standard deviation of moisture (x^w), chloride (x^{NaCl}) contents and water activity (a_w) for each sampling zone and ham type: fat (■), lean (□) and semi-fat (▨) at four post-salting times, weeks (W).

Figure 4 shows sodium concentration values in the meat liquid phase (z^{NaCl}) after 8, 10, 12 and 14 weeks of post-salting in zones A, B, C and R in the different ham types.

Table 1 shows the results obtained in the multi-factor ANOVA for each physicochemical parameter

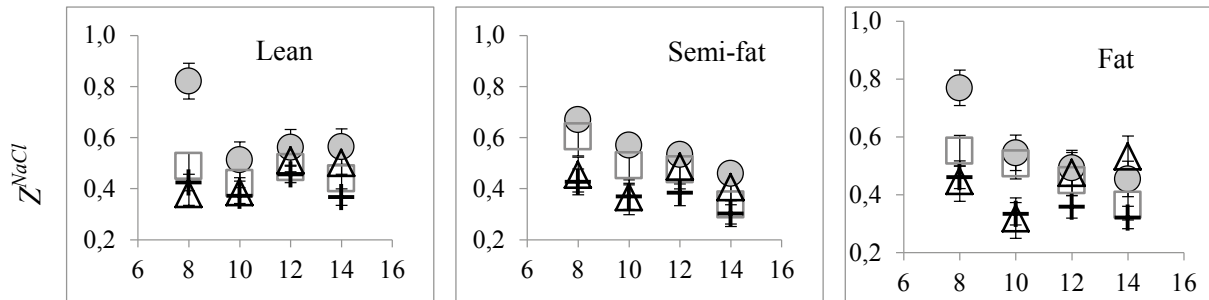


Figure 4. Mean values \pm standard deviation of sodium chloride concentration in the liquid phase (z^{NaCl}) at 8, 10, 12 and 14 post-salting weeks. Zones: A (●), B (□), C (Δ), and R (+).

Table 1. Significance level obtained in multifactor ANOVA for the physicochemical parameters according to the factors: post-salting time (A), ham type (B), sampling zone (C) and their interactions.

Factor	α			
	x^{iv}	x^{NaCl}	z^{NaCl}	a_w
A (post-salting time)	***	*	***	**
B (ham type)	ns	ns	ns	*
C (sampling zones)	***	***	***	***
A*B	ns	ns	ns	ns
A*C	**	**	***	ns
B*C	ns	ns	ns	ns
A*B*C	*	ns	ns	ns

(α): ns: no significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Electronic measurements

Figure 5 shows the average values of the impedance response were measuring the different ham types for B zone, differences between the electronic responses of the three groups of hams tested are shown in both modulus and phase.

Figure 6 shows the PCA analysis of the electronic data (modulus and phase) in A, B, and C zones at 8, 10, 12 and 14 weeks of post-salting for the different ham types.

Table 2 shows the statistics values for partial least square discriminant analysis employing all the frequencies of impedance spectroscopy and selected by iPLS algorithm at eight and ten post-salting weeks.

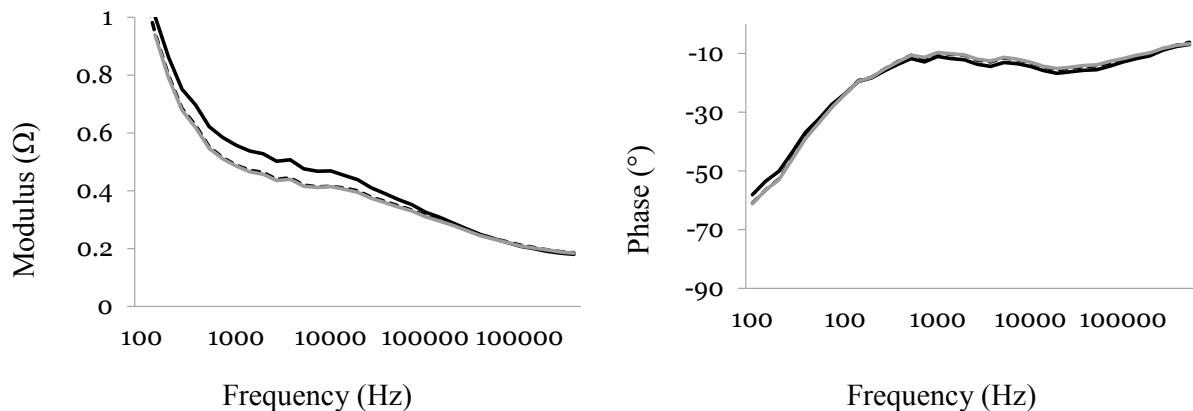


Figure 5. Bode plots for mean values for B zone four post-salting times of impedance measurements of modulus (left) and phase (right); for each ham type: fat (---), lean (-) and semi-fat (-).

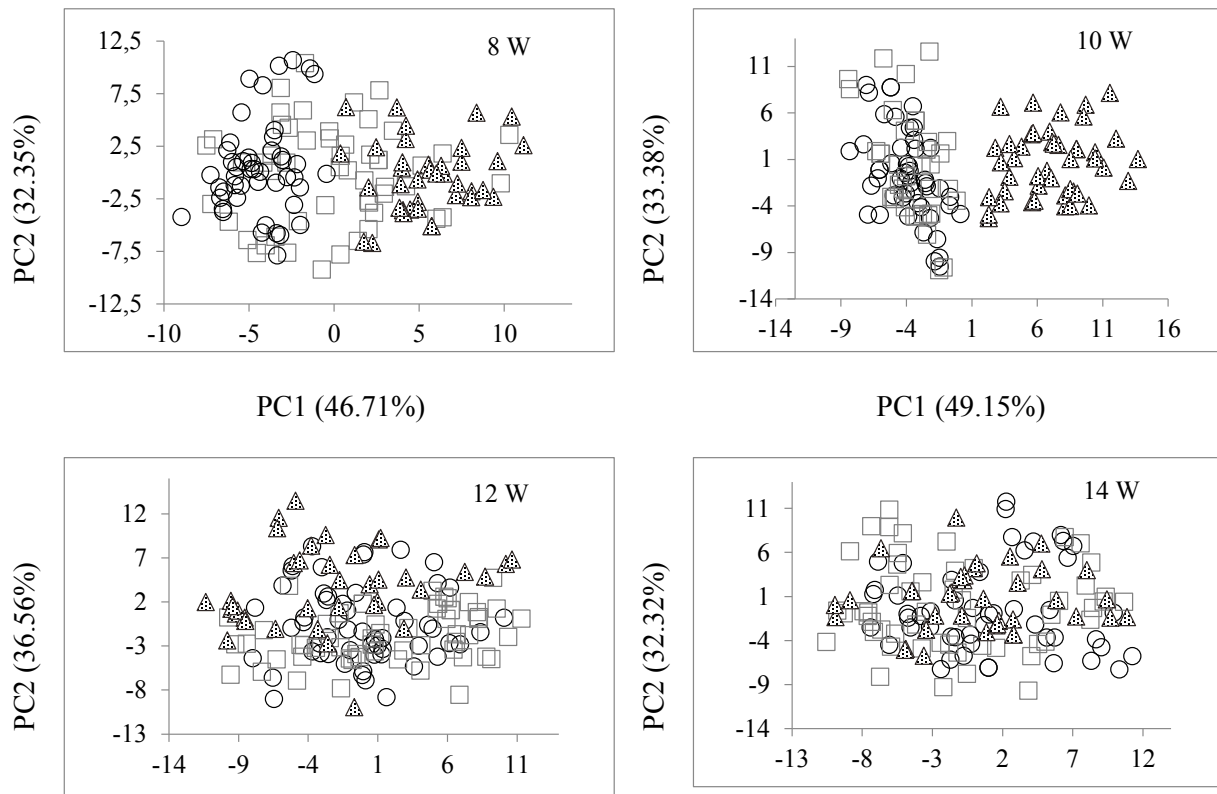


Figure 6. Principal components analysis (PCA) for the impedance signal with modulus and phase data for each post-salting time, weeks (W), zones: A (○), B (□), C (△). in A, B, C and R zones at 8, 10, 12 and 14 weeks of post-salting.

Table 2. Statistics values for partial least square discriminant analysis employing all the frequencies of impedance spectroscopy and selected by iPLS algorithm at eight and ten post-salting weeks.

Frequency	Parameter	Num. LVs	RMSEC	RMSECV	R ² Cal	R ² CV
All signal	x^{fat}	3	0.022	0.024	0.880	0.752
	x^{NaCl}	4	0.021	0.022	0.618	0.592
	z^{NaCl}	4	0.115	0.120	0.579	0.530
	a_w	2	0.009	0.009	0.832	0.731
Selection iPLS	x^{fat}	4	0.023	0.024	0.923	0.799
	x^{NaCl}	5	0.029	0.030	0.705	0.676
	z^{NaCl}	2	0.013	0.014	0.798	0.755
	a_w	4	0.009	0.009	0.891	0.773

RMSEC: Calibration Root Mean Square Error, RMSECV: Cross validation Root Mean Square Error, R²Cal: Calibration Correlation coefficient, R²CV: Cross validation Correlation coefficient.

DISCUSSION

Mass variation (ΔM_t post-salting) showed that fat hams presented smaller reduction of mass for each sampling time, compared to lean and semi-fat hams, similar values were found by other authors (19) for 50 post-salting days.

The fat content (x^f) for each ham category was confirmed. Higher values were obtained for fat and semi-fat samples. There were statistical differences ($p \leq 0.05$) between lean and fat pieces. As expected, C sampling zone presented greater fat content than B and A zones; this is because zone C is located near the subcutaneous adipose tissue.

No significant differences were found for the moisture content among the post-salting times ($p \geq 0.05$). The average moisture content (zone R) decreased with time because of the drying process (20). The time and zone factors show significant differences ($p \leq 0.05$) as well as their interaction. Moisture values between different ham types for the same sampling zone presented significant differences ($p \leq 0.05$). The external zone (A) that is directly exposed to salt, and therefore its water content dropped rapidly (21) presenting the greatest variations. Values obtained for the salt content (x^{NaCl}) were similar to those presented by other authors (22, 23). There were significant differences ($p \leq 0.05$) between the sampling areas after the eighth and tenth post-salting weeks. These differences were reduced over time because of the diffusion process (24). Salt concentration at fourteenth week was same for all sampling zones. No significant differences were found for ham types ($p \geq 0.05$). In terms of a_w hams presented the same behavior throughout the post-salting step. Sampling time, ham type and sampling zone influenced the water activity values ($p \leq 0.05$); factors interaction did not present significant differences ($p \geq 0.05$). Regarding salt concentration in liquid phase (z^{NaCl}) (Figure 4), post-salting time and zone, and their interaction, were statistically significant ($p \leq 0.05$).

During the post-salting step the values of z^{NaCl} and a_w tend to be equal due to the salt transfer from the surface to the inner part of the ham, which is mainly due to the evaporation of the water, which allows salt uptake and changes in the moisture content (25, 26). At the end of post-salting step (fourteen week) no significant differences ($p \geq 0.05$) were observed between sampling zones and ham types. Therefore, processing conditions for the tray salting and post-salting steps were correctly performed; nevertheless, a drying step is needed in order to obtain further reduction of moisture and a_w values to ensure a good product.

Electronic characterization.

Electronic response (Figure 5) obtained for modulus showed a different signal for the lean hams compared to fat and semi-fat hams, the range of frequencies between 100 and 10000 Hz presented the highest difference for lean hams, differences diminished when comparing the phase measurements for the samples, similar results were found for A and C zones. The statistical analysis for the impedance signals showed a discrimination accor-

ding to the sampling zones (A, B, C). However, the sensor was not able to classify the samples base on the ham type. Depending on the post-salting time, the signal could generate different groups. For eight and ten weeks (Figure 4) measurements of zone A were located on the left side of component one (46.71 and 49.15%), while measurements of zone C were located to the right side. At twelve and fourteen post-salting weeks, the discrimination observed in the previous weeks was reduced to disappear. The changes on the signal are also similar to those occurred on the physicochemical parameters that tends to an equilibrium on salt and moisture content between the sampling zones at the end of the post-salting (11). In order to explain the observed differences of the signal for eight and ten weeks, a PLS regressions were performed between the electronic information and the physicochemical parameters (Table 2). Moisture content ($R^2CV x^w = 0.75$) and the water activity ($R^2CV a_w = 0.73$) presented better statistical description, this fact agrees with other authors who had already studied the impedance spectroscopy (14, 27). With the purpose of improving the study, an iPLS selection (100 to 1000 Hz) was performed (28). All the physicochemical parameters presented better cross validation results. Moisture content ($R^2CV x^w = 0.79$) and water activity ($R^2CV a_w = 0.77$) shown the best performance from the evaluated parameters (Table 2). The impedance spectroscopy technique generated different signals between the zones of analysis during the first two post-salting times, eight and ten weeks, in which physicochemical differences were also observed as a consequence of the variation in the salt concentration between zones. However, it is necessary to increase the research in this area to improve the obtained results, in order to develop a sensor to control the dry-ham manufacturing process and guarantees the consumers safety.

CONFLICT OF INTEREST

The authors certify that there is no conflict of interest in this research.

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AUTHORS' CONTRIBUTIONS

Conception and design of study: J Barat B, R Grau M, J Girón H, R Masot P; acquisition of data: J Girón H; analysis and/or interpretation of data: J Barat B, R Grau M, J Girón H, R Masot P.

REFERENCES

- Hui Y H. Handbook of Meat and Meat Processing, Second Edition. Boca Raton (Unites States of America), 2012.
- Aliño M, Grau R, Toldrá F, Barat J. Physicochemical changes in dry-cured hams salted with potassium, calcium and magnesium chloride as a partial replacement for sodium chloride. *Meat Sci* 2010; 86: 331-336.
- Santos-Garcés E, Muñoz I, Gou P, García-Gil F, Fulladosa E. Including estimated intramuscular fat content from computed tomography images improves prediction accuracy of dry-cured ham composition. *Meat Sci* 2014; 96(2A): 943-947.
- Pérez-Palacios T, Caballero D, Caro A, Rodríguez P, Antequera T. Applying data mining and Computer Vision Techniques to MRI to estimate quality traits in Iberian hams. *J Food Eng* 2014; 131: 82-88.
- Caballero D, Caro A, Rodríguez P, Durán M, Ávila M, Palacios R, Antequera T, Pérez-Palacios T. Modeling salt diffusion in Iberian ham by applying MRI and data mining. *J of Food Eng* 2016; 189: 115-122.
- Bajd F, Škrlep M, Čandek-Potokar M, Vidmar J, Serša I. Use of multiparametric magnetic resonance microscopy for discrimination among different processing protocols and anatomical positions of Slovenian dry-cured hams. *Food Chem* 2016; 197(B): 1093-1101.
- Tian-Hao C, Ye-Pei Z, Min-Yi H, Peng W, Ran W, Xing-Lian X, Guang-Hong Z. Classification of chicken muscle with different freeze-thaw cycles using impedance and physicochemical properties. *J Food Eng* 2017; 196: 94-100.
- Damez L, Clerjon S, Abouelkaram S, Lepetit J. Electrical impedance probing of the muscle food anisotropy for meat ageing control. *Food Control* 2008; 19(10): 931-939.
- Wu L, Ogawa Y, Tagawa A. Electrical impedance spectroscopy analysis of eggplant pulp and effects of drying and freezing-thawing treatments on its impedance characteristics. *J Food Eng* 2008; 87: 274-280.
- Vadim FL. Impedance Spectroscopy: Applications to Electrochemical and Dielectric Phenomena. John Wiley & Sons, Inc. (Unites States of America), 2012.
- Aliño M, Grau R, Fuentes A, Barat J. Influence of low-sodium mixtures of salts on the post-salting stage of dry-cured ham process. *J Food Eng* 2010; 99(2): 198-205.
- AOAC. Official Methods of analysis (20th edition). Association of Official Analytical Chemists, Arlington (United States of America), 2016.
- Girón J, Gil-Sánchez L, García-Breijo E, Pagán M, Barat J, Grau R. Development of potentiometric equipment for the identification of altered dry-cured hams: A preliminary study. *Meat Sci* 2015; 106: 1-5.
- Masot R. Tesis Doctoral: Desarrollo de un sistema de medida basado en espectroscopia de impedancia para la determinación de parámetros físicoquímicos en alimentos. Universidad Politécnica de Valencia. 2010.
- Pérez-Esteve E, Fuentes A, Grau R, Fernández-Segovia I, Masot R, Alcañiz M, Barat J. Use of impedance spectroscopy for predicting freshness of sea bream (*Sparus aurata*). *Food Control* 2014; 35(1), 360-365.
- Rizo A, Fuentes A, Fernández-Segovia I, Masot R, Alcañiz M, Barat J. Development of a new salmon salting-smoking method and process monitoring by impedance spectroscopy. *LWT-Food Sci Technol* 2013; 51(1): 218-224.
- Fuentes A, Masot R, Fernández-Segovia, Ruiz-Rico M, Alcañiz M, Barat J. Differentiation between fresh and frozen-thawed sea bream (*Sparus aurata*) using impedance spectroscopy techniques. *Innov Food Sci Emerg Technologies* 2013; 19: 210-217.
- Grau R, Sánchez J, Girón J, Ivorra E, Fuentes A, Barat J. Non-destructive assessment of freshness in packaged sliced chicken breasts using SW-NIR spectroscopy. *Food Res Int* 2011; 44(1): 331-337.
- Armenteros M, Aristoy C, Barat J, Toldrá F. Biochemical and sensory changes in dry-cured ham salted with partial replacements of NaCl by other chloride salts. *Meat Sci* 2012; 90(2): 361-367.
- Pérez-Palacios T, Antequera T, Durán M, Caro A, Rodríguez P, Palacios R. MRI-based analysis of feeding background effect on fresh Iberian ham. *Food Chem* 2011; 126(3): 1366-1372.
- Andronikov D, Gasperlin L, Polak T, Zlender B. Texture and quality parameters of Slovenian Dry-Cured Ham *Kraški prsut* according to mass and salt levels. *Food Technol Biotech* 2013; 51(1): 112-122.
- Benedini R, Parolari G, Toscani T, Virgili R. Sensory and texture properties of Italian typical dry-cured hams as related to maturation time and salt content. *Meat Sci* 2012; 90(2): 431-437.
- Martínez-Onandi N, Rivas-Cañedo A, Picon A, Nuñez M. Influence of physicochemical parameters and high pressure processing on the volatile compounds of Serrano dry-cured ham after prolonged refrigerated storage. *Meat Sci* 2016; 125: 101-108.
- Grau R, Albarracín W, Clemente G, Barat J. Study of the influence of product process variables in the salting and post-salting stages of PSE thawed hams. *International J Food Eng* 2007; 3(5): 11-16.
- Pugliese C, Sirtori F, Škrlep M, Piasentier E, Calamai L, Oreste F, Čandek-Potokar M. The effect of ripening time on the chemical, textural, volatile and sensorial traits of Bicep femoris and Semimembranosus muscles of the Slovenian dry-cured ham *Kraški prsut*. *Meat Sci* 2015; 100: 58-68.
- Comaposada J, Gou P, Arnau J. The effect of sodium chloride content and temperature on pork meat isotherms. *Meat Sci* 2000; 55(3): 291-295.
- García Breijo E; Barat J; Torres O; Gil-Sánchez L; Ibáñez J; Alcañiz M, Masot R, Fraile R. Development of a puncture electronic device for electrical conductivity measurements throughout meat salting. *Sensor Actuat A-Phys* 2008; 148(1): 63-67.
- De Jesús C, Hernández-Coronado G, Girón J, Barat J, Pagán M, Alcañiz M, Masot R, Raúl G. Classification of unaltered and altered dry-cured ham by impedance spectroscopy: A preliminary study. *Meat Sci* 2014; 98(4): 695-700.