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Classification of unaltered and altered dry-cured ham by impedance spectroscopy: A preliminary study

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ABSTRACT

The aims of this work were characterized by the physicochemical and microbiological features of dried-cured hams classified by the manufacturer as altered (deep spoilage and swollen) and unaltered, as well as, correlated these results with the electronic measurements of impedance spectroscopy, in order to lay the groundwork to design a suitable electrode to be used for checking in line all dry-cured ham elaborated. Double electrode and coaxial needle electrode were used in a frequency range of 100 Hz to 1 MHz. The electronic measurements of the two electrodes were able undoubtedly to discriminate between altered and unaltered dry-cured hams; moreover a tendency to classify between deep spoilage and swollen hams was shown. The values into the range of frequencies between 100 Hz–1000 Hz, for both electrodes, showed the best clustering results. More studies are needed to select the best electrode which can be transformed in a robust and versatile electrode which could be used for checking in line all dry-cured ham elaborated.

1. Introduction

Deep spoilage also known as bone taint is an alteration present in dry-cured ham, appearing close to the bone structure in the large pieces of muscles and is mainly defined by off-odor and its noticeable pasty texture, which could affect either the whole piece or just a specific zone (Martín et al., 2008; Paarup, Nieto, Peláez, & Reguera, 1999). The change of the texture is the result of the proteolytic activity with either enzymatic or microbial origin (Martín et al., 2010), even so microorganisms are the main reason related with this defect, among the most remarkable are *Clostridium*, *Staphylococcus*, *Micrococcus*, Enterobacteriaceae such *as Serratia*, *Proteus*, *Leclercia* and *Hafnia*; and finally at a lower concentration lactic acid bacteria (Losantos, Sanabria, Cornejo, & Carrascosa, 2000; Martín et al., 2008; Paarup et al., 1999). These microorganisms generate small molecules from meat proteins, such as organic acid, which are responsible of generating unacceptable off-flavors (Martín et al., 2008). Some genera of Fungi are also present in this meat alteration such as *Cladosporium*, *Aspergillus*, *Eurotium*, *Alternaria* or *Penicillium* producing other kinds of changes such as pigmentation and unpleasant odors (Núñez, Rodríguez, Bermúdez, Córdoba, & Asensio, 1996). From methionine and cysteine either by microbial or enzymatic via, hydrogen sulfide and methyl mercaptan are formed, whereas low concentrations of these compounds are related to the aroma of dry-cured ham, these gasses are the main responsible of the formation of internal pockets (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008), which are detected by the sound made when the piece is hit, this alteration is known as swollen dry-cured ham (Losantos et al., 2000).

Traditional techniques of analysis can be replaced with the use of biosensors at a low cost, quick response, and with the possibility to be applied to a widely range of samples, with a high specificity and simplicity (Mello & Kubota, 2002). When the resistance of a material to the flow of an electric current is frequency dependent is called impedance, with a real part called resistance and other imaginary know as capacitance (Damez & Clerjon, 2013; Damez, Clerjon, Abouelkaram, & Lepetit, 2008a). The name of bioimpedance, is given in the case of biological tissues, in which case the working frequencies are below 1 MHz, because the cellular membrane is non-conductive and the intracellular fluid work as an electrolyte, increasing the capacitance by inducing an electric current in this frequency values (Damez & Clerjon, 2013), this behavior is frequently represented using the Fricke model (Damez, Clerjon, Abouelkaram, & Lepetit, 2008b).

Among the main factors related with the increase of the uncertainty of the measurements are sample composition, physiological condition of the tissue, anisotropy and polarization of the electrodes (Gabriel, Peyman, & Grant, 2009). Electrode polarization show the organization of the molecular charges in the sample-electrode interface, in a simplified form can be modeled as a parallel combination of a capacitor and a resistor, although in tissues is more complicated, due to the changes provoke by the electrode puncture, which are the liberation of the

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adjacent electrolyte to the puncture point and the decrease of the conductivity by the structural damage (Miklavcic, Pavselj, & Hart, 2006).

Bioimpedance is also affected by the tissue anisotropy, referred to the interaction between electrode and the orientations of the main axis of the sample, which can be longitudinal, transversal or a combination of both, in the case of muscles is easier conduct an electric current in longitudinal direction than in transversal manner. Even though anisotropy depends on the frequency, in range of megahertz anisotropy disappears (Damez et al., 2008b; Gabriel et al., 2009; Miklavcic et al., 2006).

The electrodes in bioimpedance could be configured in two ways, the first is the method which involves two electrodes and is also called bipolar, and is the most basic and common, in this system alternating current is only applied and a systematic error is produced due to the polarization reached at low frequencies (Damez & Clerjon, 2013; Miklavcic et al., 2006); and the second method is the tetrapolar, where two pairs of electrodes are used, one pair internal and the other external; direct or altering current could be applied, in this case the polarization of the internal electrodes is minimal, the election of the bipolar system with an error correction is recommended by Miklavcic et al. (2006). Even though in the tetrapolar system the polarization of the internal electrodes is almost zero it is not possible to eliminate it. Based on the foregoing, the aims of this work were to characterize the physicochemical and microbiological features of dried cured hams classified by the manufacturer as altered (deep spoilage and swollen) and unaltered, as well as, correlated these results with the electronic measurements obtained from the impedance spectroscopy equipment in order to lay the groundwork to design a suitable electrode to be used for checking in line all the dry-cured hams elaborated.

2. Materials and methods

2.1. Selection of the dry-cured hams

Seventeen dry-cured hams with an average weight of 8.0 ± 0.5 kg, manufactured according to the traditional method, were obtained from a dry-cured ham manufacturer. These hams were classified according to workers' experience into three groups: the first was defined as unaltered form by six hams (U); the second group was presented as deep spoilage (D) by six hams and lastly the group of spoiled swollen hams (S) with five hams.

2.2. Sampling procedure

Sampling was carried out in three different zones which are shown in Fig. 1. M1 corresponding to the closest zone to the coxofemoral junction, M2 located at the farthest zone to the coxofemoral junction and B situated at the farthest zone to the main joint and close to the hoof, near the tibia and the fibula.

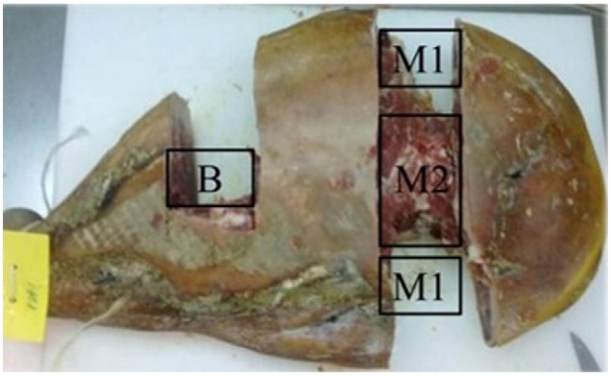

Fig. 1. Sampling zones B, M1 and M2.

2.3. Analytical determinations

The analysis was done in triplicate on each sample, except for pH, which was measured five times.

2.3.1. Physico-chemical analyses

Moisture content was determined by the AOAC (1997). Sodium chloride was determined after sampling homogenization in approximate 40 mL distilled water at 9000 rpm with ULTRATURRAX T25 (Janke & Kundel, Staufen, Germany) for 5 min. Next, distilled water was added to complete the 100 mL of solution, and then was filtrated. An aliquot of this solution was taken to determine sodium chloride content (mg/L) with an automatic Chloride Analyzer (Sherwood Scientific Ltd., Cambridge, UK). Water activity (a_w) was measured in minced samples with a fast water activity-meter (GBX scientific Fast/1, Cédex, France). The pH of the hams was measured with a portable pH meter micropH 2001 (Crison Instruments, S.A., Barcelona, Spain) with a puncture electrode (Crison 5231) in five different locations of the sample.

2.3.2. Microbiological analysis

From each ham, 10 ± 1 g of the sample was taken aseptically and homogenized in stomacher (IUL Instruments, Barcelona, Spain) for 1 min, with 90 mL of sterile peptone solution. Further decimal dilutions were made conveniently with sterile peptone solution. Moreover the microbial counts were obtained performing plate counts of the different microbial groups of interest: aerobic mesophilic flora, determined by the method given by UNE-EN ISO 4833: 2003 standard (ISO, 2003), incubating for 72 h at 30 ± 1 °C in Plate Count agar; aerobic mesophilic salt tolerant flora, in Plate Count Agar (PCA) with 10% w/v of NaCl after incubating for 72 h at 30 ± 1 °C (Tomlinson, 1995); Enterobacteriaceae, enumerated in Violet Red Bile Glucose agar after incubating 24 h at 37 ± 1 °C (ISO, 21528-2/2004). Micrococcaceae, determinated in manitol salt agar (MSA) incubating for 48 h at 37 ± 1 °C as described in a previous work (Cordero & Zumalacárregui, 2000). Lactic acid bacteria, in De Man, Rogosa and Sharpe Agar (MRS) incubated at 37 ± 1 °C for 48 h (ISO, 15214/1998). Sulfite-reducing bacteria determined in sulfite polymyxin sulfadiazine agar (SPS) at 47 ± 1 °C for 48 h (Tomlinson, 1995). Molds and yeasts in Chloramphenicol Glucose agar (CGA) after 5 days at 25 ± 1 °C (ISO 7954/1987). All media and reagents were provided by Scharlau Chemie, S.A., Barcelona, Spain. Microbial counts were obtained by duplicate and all the results were expressed as logarithm of colony-forming units per gram (log CFU/g).

2.4. Impedance spectroscopy measurements

Electronic measurements were performed in the solid matrix of the dry-cured ham by inserting the sensors into the sample. The penetration depth of electrodes was kept constant at 1.5 cm in all measurements and determinations were made by triplicate at room temperature (~25 °C). Although the phenomena of anisotropy could be present in the measurements, these were taken regardless of the fiber orientation in order to obtain a robust device to be used for checking in situ.

2.4.1. Impedance equipment

A low-cost, flexible, light, non-destructive measurement system was developed by the "Institute of Molecular Recognition and Technological Development (IDM)" at the Universitat Politècnica de València (UPV). This impedance spectroscopy measurement system applies an electric signal to the sample and measures the response in a frequency sweep between 1 Hz and 1 MHz, generating a sinusoidal signal, with an amplitude of up to 1 Vpp. The system consists of a software application that runs on a PC and an 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. With the current and voltage from the electrodes a Fourier analysis is