ESRF	**Experiment title: Determination of Iberian Cured Ham Process by Characterization of Myoglobin-Metal Species as Biomarkers by XAS Techniques.**	**Experiment number**: 25-02-705
Beamline: BM-25A	**Date of experiment**: from: 13/06/2010 to: 15/06/2010	**Date of report**:
Shifts: 6	**Local contact(s)**: Pilar Ferrer Escorihuela	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*[1]Manuel Valiente (Main Proposer)

[1]Diego Morillo

[1]Francisco Blanco

[2]Silvia Serranti

[1]Centre GTS, Department of Chemistry, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain
[2]Dipartimento di Ingegneria Chimica Materiali Ambiente, Sapienza – Università di Roma, 00184 Roma, Italy.

Report:

Cured ham process compresses many different biochemical reactions, resulting in a complex process, which has often been studied. It is accepted that the colour changes affecting cured ham are due to myoglobin metal complexes including Fe and Zn species. Although some hams are treated with nitrites, in order to simulate high quality products, the use of such compounds is strictly forbidden for the treatment of Parma (Italy) and Jabugo (Spain) high quality ham. In this sense, the main aim of this study is the characterisation of the species mainly involved in this process, since they can give key information for the determination of the quality of the ham [1,2].

In this experiment, ham samples from Parma and San Danielle (Italy) and from Jabubo (Spain) were analysed. For the Spanish ones, up to three different curing ages were studied: years 2007, 2008 and 2009.

The samples taken into the synchrotron were analysed by means of XANES technique at BM25A. Two different energy ranges were studied, namely Fe K-edge (7112 eV) and Zn K-edge (9659 eV). A calibration of the energies, to determine the channels able for the measurement of each element, was done.

Some standards are measured, so as to calibrate the energies of the edge positions for Fe and Zn in different environments. The molecules used as standards are the complexes Fe/Zn with myoglobin and porphyrin, as well as their mixtures. They represent, actually, the chemical environments where the analysed metals are mainly found in the ham. They were analysed on transmittance mode, by making pellets. On the other hand, real samples of 2-3 mm thick were directly used for its analysis. A little part of a slice of ham, containing no fat, was cutted and covered with Capton, to fix it to the sample holder. Due to the rather low metal concentration in the samples, the analyses were performed using a fluorescence detector.

As shown in Figure 1, the edge energy for different complexes of the same metal differs. This phenomenon might be understood the degradation of biological molecules when applying energy is considered, as already reported in literature.

For the analysis of the samples, a scan at 20 keV was done on every sample. With this scan it was possible to define the positions on each sample where Fe and Zn were found with a highest concentration. The program PyMCA was used for this purpose, obtaining an image as seen in Figure 2.

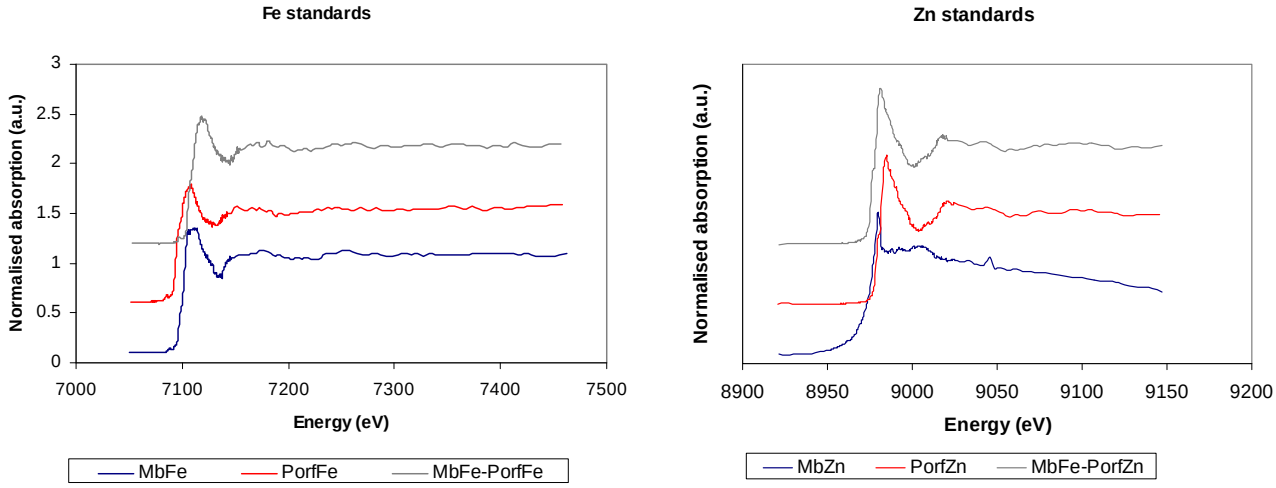

Figure 1. Standards for the metal complexes. The metal complexes for two ligands were studied, as well as their mixture. Mb, Mioglobyn; Porf, Porphyrin.

With a general map of concentrations of the sample, an XAS analysis was performed on two different points for every sample. The criterion for choosing these points was the aim of taking the highest concentration of Fe and Zn, to record the best XAS spectrum possible. Therefore, two spectra (one for Fe and another one for Zn) were obtained at every point for each sample.

An example of this procedure is provided for a ham sample from Jabugo, 2007.

2007

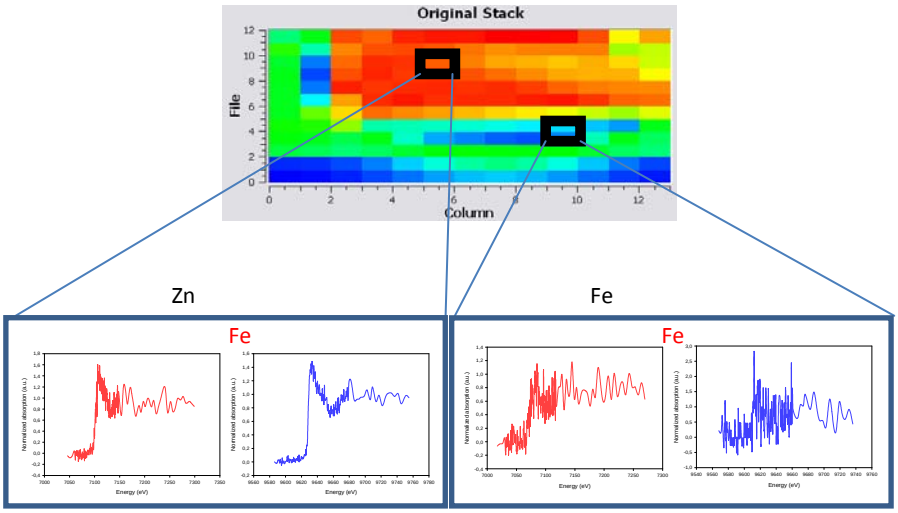

Figure 2. PyMCA image, where the regions of the sample of highest content for Zn and Fe (Zn in this picture) were selected. For every point, the XAS spectra for Zn and Fe was measured.

As it can be seen in Figure 2, the XAS spectra for the metals analysed during the experiment are noisy and, in some cases, the edge can hardly be detected. For that reason, the XANES analysis could not be performed correctly on many samples. Logically, XAFS analysis could not be carried out on these samples, due to the noise in the spectra.

Therefore, the performance of this experiment in liquid nitrogen conditions is suggested, in order to prevent sample degradation. A new beamtime for the continuation of this experiment has been allocated, experiment CH-3321. Since the use of liquid nitrogen for the cryoanalysis of the samples is feasible on BM25A, we propose this new methodology for the acquisition of better defined spectra, which would lead to the understanding of the system.

[1] C.E. Adamsen, J.K.S. Møller, G. Parolari, L. Gabba, L. H. Skibsted. *Changes in Znprophyrin and proteinous pigments in italian dry-cured ham Turing processing and maturation.* 2006. Meat Science 74, 373-379.

[2] G. Lindahl, K. Lundströmb, E. Tornberg. *Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs.* 2001. Meat Science, 59(2), 141-151.