ESRF	**Experiment title**: **Determination of Iberian and Italian Cured Ham Process by Characterization of Myoglobin-Metal Species as Biomarkers by XAS Techniques**	**Experiment number**: CH-3321
Beamline: BM25A	**Date of experiment**: 01-05/07/11 and 25-30/09/11	**Date of report**:
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Report:

It is well known that the colour change observed during the curing process of ham samples is due to an ion exchange in some proteins. The mechanism whereby the Fe ion in the porphyrin ring of the myoglobin is exchanged by Zn ion is not well defined, though. Nonetheless, the curing process may have a strong dependence on this reaction. The change in ham colour is sometimes simulated in low quality hams, but this treatment is absolutely forbidden in high quality hams such as Jabugo (Spain) and Parma (Italy). For these purposes the use of X-ray synchrotron radiation (XAS technique) has been suggested.

For the study of these process, a first experiment (25-02 705) was performed at ESRF – Beamline BM 25. During this first experiment, similar samples (Spanish and Italian hams, with three different curing times for the former one) as the ones measured during the experiment CH-3321 were studied. The conditions used for the measurements together with the rather low concentration of the metals studied in the sample led to the recording of noisy spectra. Thus, a follow-up experiment was suggested.

In the experiment CH-3321, XAS measurements of several ham samples were done. Three different curing times (1, 2 and 3 years) were analysed for Jabugo ham (Spain), while in the case of Italian samples, 16 (Parma) and 20 (San Daniele) months of curing time were considered. The spectra were recorded on the K-edge regions of Fe (7112 eV) and Zn (9659 eV). A calibration of the energy was done, so as to determine the most suitable channels for the measurement of each element. The corresponding standards were also analysed, namely Fe and Zn porphyrin complexes. While the standards were taken into the synchrotron as pellets, the samples were analysed by cutting 1 cm^2 squares from ham slices of 1 mm thickness. In every case, samples were kept into Kapton. The standards were measured in transmission mode, whereas for recording the spectra on ham samples a 13 ROI fluorescence detector was used, due to low concentration of the studied metals.

In addition, in order to improve the signal-to-noise ratio of the spectra, cryogenic conditions where also tested. The temperature around the sample environment was dropped down to about -80˚C constantly flushing liquid nitrogen on the sample holder.

In order to determine which part of the samples had a higher amout of both metals of study, mapping experiments were performed. The surface of the sample was divided into a 9x9 grid, in every piece of which a EDS scan applying 20 keV was measured. Then, the ratio for Zn/Fe concentration was calculated, to assess the best positions on which the XAS scans should be measured.

The results show some interesting features. In regard to the cryogenic conditions, although it is generally accepted that low temperature allows recording of spectra with a higher S/N ratio, especially in biological samples, this improvement was not noted in our case (Fig.1). Therefore, all the spectra showed were recorded at room temperature.

An example of the mapping experiments performed on the samples can be seen in Fig. 2. The areas with a highest and lowest ratio Zn/Fe was analysed, as well as an area where this ratio was constant.

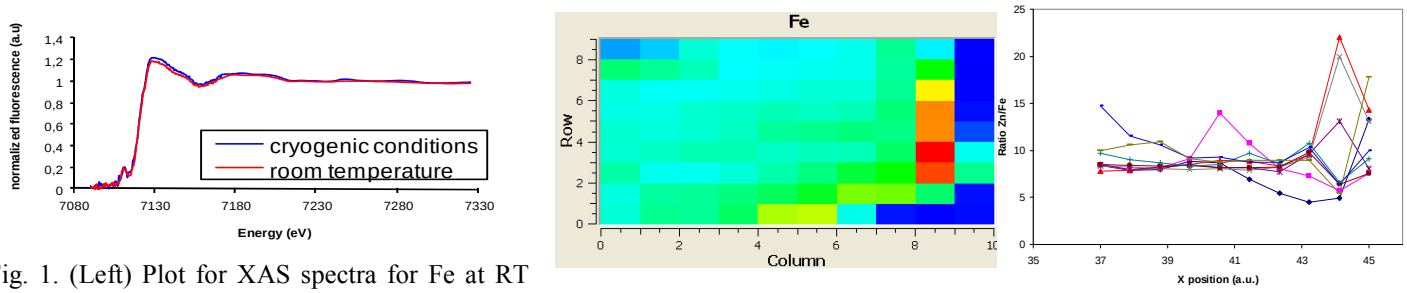

Fig. 1. (Left) Plot for XAS spectra for Fe at RT and cryogenic conditions. Fig. 2. (Center and Right) Intensities for the mapping of a sample (center) and the Zn/Fe ratio (right). In the plot at the right side, each colour stands for a row of pixels.

The XAS measurements performed on the samples show less noise than those recorded in the previous experiment (25-02 705). Despite the improvement in the measurements, in the case of Fe the spectra are still too noisy for a really precise definition of the edge, due to the low concentration of Fe in every case. Nonetheless, it can be appreciated (see Fig.3) that the environment for Fe is similar in all the samples. By contrast, much better defined spectra could be measured in the case of Zn. It is worth to highlight the finding of absolutely different Zn spectra in some samples of ham, as shown in Fig. 4. Not only the peak is shifted from the K edge seen for the standard, but also the shape of the peak varies. This fact may suggest different environments for the Zn ion even in the same sample.

Additionally, a comparison between different origins of ham has been done. It can be appreciated in Fig. 5 how the K-edge position is slightly shifted when Spanish and Italian hams are compared.

The comparison between different curing times also shows a slight difference between several samples of Spanish ham, though this feature needs deeper analysis in order to get a clear picture of the undergoing process.

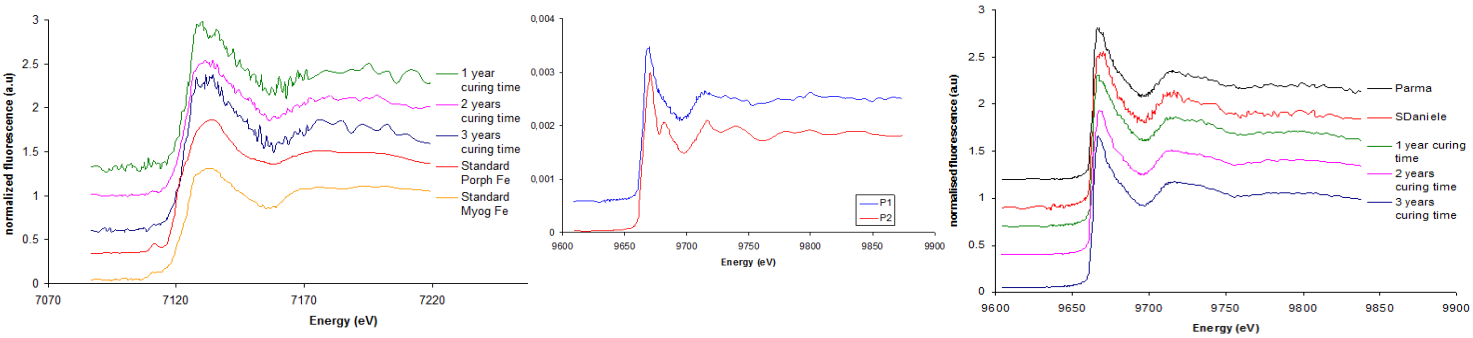

Fig. 3. (Left) Fe spectra for Spanish ham. Fig. 4. (Center) Zn spectra for two different points in the same sample; a shoulder appears in one case, sign of a substantially different environment for the ion. Fig.5 (Right) Zn Spectra comparing Spanish and Italian hams.

Table 1. Quantification of metals

FP-XRF Quantification of metals			
Sample		Fe (ppm)	Zn (ppm)
Spanish (Jabugo)	1 year	<15	77 ± 5
	2 years		82 ± 4
	3 years		65 ± 4
Italian	San Daniele		93 ± 5
	Parma		110 ± 5

Table 1 shows the quantification of metals in the samples, performed by FP-XRF. It can be seen that the concentration of Fe is really low, fact that explains the noisy spectra that are recorded. By contrast, Zn concentrations are fairly high, which allows to record better spectra.

To sum up, these results show promising features for the analysis of ham. While the measurement of Fe XAS spectra appears to be somewhat knotty, mostly due to the low concentration measured in ham samples, the Zn spectra show evidence for some differences in the ion environment. These results reasonably suggest Zn to be a better biomarker than Fe for the purposes of the present work.

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