EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** FTIRM as a tool to evaluate protective effect of phenolic compounds against oxidative stress in enterocytes	**Experiment number**: LS-2445
Beamline: ID-21	**Date of experiment**: from:November 16, 2015 to: November 20, 2015	**Date of report**:
Shifts: 12	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Laura A. A. De la Rosa Carrillo (Universidad Autónoma de Ciudad Juárez)
Dr. Emilio Álvarez Parrilla (Universidad Autónoma de Ciudad Juárez)
M.S. Guillermo A. Barraza Garza (Universidad Autónoma de Ciudad Juárez) *

Report:

LS 2445 proposal had as objective the study of the protective effect of different polyphenols: catechin (Cat), quercetin (Qc) and capsaicin (Cap), against H_2O_2-induced oxidative stress evaluated in rat enterocytes using Fourier Transform Infrared Microspectroscopy (FTIRM) at ID-21. Results were compared to standard lipid peroxidation techniques: conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS). Analysis of FTIRM spectral data allowed the simultaneous evaluation of the effects of H_2O_2 and polyphenols on lipid and protein oxidation. All polyphenols showed a protective effect against H_2O_2-induced oxidative stress in enterocytes, when administered before or after H_2O_2. Catechin and capsaicin showed the highest protective effect, while Quercetin presented a mild pro-oxidative effect (biochemical profile between control and H_2O_2-treated cells) according to FTIR analyses. These results demonstrated the viability to use infrared spectroscopy to evaluate the oxidant and antioxidant effect of molecules in cell systems assays.

Objectives:

- Observe protective effect of polyphenols using the cell line IEC-6 as model.
- Determine whether the protective effect of polyphenolic compounds generated in cells subjected to oxidative stress is concentration dependant or treatment dependant.
- Obtain infrared spectra of individual cells and infrared imaging.

To complete the objectives an experiment was conducted, in which the cell line IEC-6 was used. These cells were subjected to treatment with H_2O_2 as oxidant and three polyphenolic compounds (capsaicin, catechin and quercetin) were used at three different concentrations before and after H_2O_2-treatment. FTIR and FTIR mycroscopy were used to analyze changes in the biochemical profiles of treated cells.

The IEC-6 cells were grown to confluence in DMEM medium to a number of 50,000 cells per well in growth plates where they were left for 24 hours to allow adherence to the plate wells. Different treatments were applied to the cells: control (DMEM medium), H_2O_2 (0.5 mM for 30 minutes), and polyphenols in 3 different concentrations (0.05 mM, 0.1 mM and 0.25 mM) in addition to DMEM medium for 30 minutes, before and after addition of H_2O_2 treatment. After treatments, cells were washed with PBS and trypsinized. Then cells

were washed with PBS and centrifuged for 5 minutes at 201 g. After washing the cells were fixed with 4% paraformaldehyde solution in PBS for 20 minutes. Fixed cells were mounted on BaF_2 windows and left to dry at room temperature. Once dried, the samples were placed for observation in the infrared microscope (FTIRM) where they were observed with an infrared beam in transmission mode.

Data collection began with the collection of infrared spectra from individual cells mounted BaF_2 windows. However the first attempts to collect data generated a large number of spectra with a faulty signal, due to "Mie scattering", a phenomenon caused by reflection of the infrared beam in organelles and membranes of the cell itself. To reduce this phenomenon, the intensity of the light source used was decreased and making measurements by linear maps in the cell allowing to get better spectra in a more efficient way. An approximate number of between 5 and 7 Infrared spectra per cell were collected.

The data collected was obtained using the following measurement parameters:

Beam size: 9 µm; Spectral resolution: 6 cm^{-1}; Number of scans per spectrum: 100 scans

Step size in linear map: 4 µm

Besides obtaining individual spectra a series of infrared maps featuring groups of cells under different conditions were obtained, which allow us to observe the same phenomena as in the individual spectra but in a group of cells and how the biochemical profiles change within the cell and how they vary between each of the treatments.

The data collected were obtained using the following measurement parameters:

Beam: 9 µm; Spectral resolution: 6 cm^{-1}; Number of scans per spectrum: 100 scans

Step size in map: 4 µm

The results are still under analysis process. Raw spectra show that there are clear differences between the different treatments in different chemical bonds. Some of this differences are related to the intensity of some bands like the ones corresponding to phosphodiester bonds (1230 cm^{-1}) and aldehyde bond (1740 cm^{-1}). Modification on the intensities of amide bands I and II were also observed. These results were simmilars to those obteined using ATR-FTIR, but the use of synchrotron source increased the signal to noise ratio, obtaining better results.

The obtained infrared maps of individual cells showed significant differences between treatments. The major observed differences are realated to lipid peroxidation. Map of control cells and H_2O_2-treated cells showed large changes at 1740 cm^{-1} which corresponds to the aldehyde bond. As is shown in figure 1, H_2O_2-treated cells have a strong absorption in this band indicating the presence of aldehydes, while control cells showed low signal at this wavenumber. Aldehydes are common products of lipid peroxidation and typically exhibit absorption in this band. The strenght of this signal arise during lipid peroxidation. Polyphenolic-treated cells showed intermediate maping pattern, indicating protective effect against H_2O_2-oxidative effect.

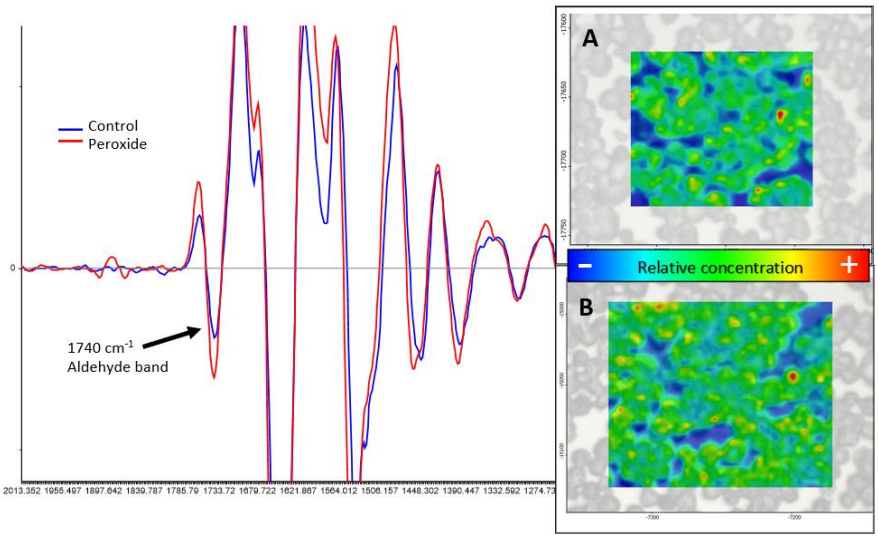

Figure 1. Second derivative spectra shows absorption in the range 2013 cm^{-1} to 1270 cm^{-1} band. The band 1740 cm^{-1} (aldehy band) show a difference in absorption between the control cells and the H_2O_2-treated cells. This difference is also seen in the IR image mapping where the relative concentration of this band is lower in control cells (A) when compared to H_2O_2-treated cells (B).