ESRF	**Experiment title:** Selenium speciation in wheat flour by XAS	**Experiment number**: 25-01-702
Beamline: BM25 Spline	**Date of experiment**: from: 10/05/2009 (08:00 a.m.) to: 14/05/2009 (08:00 a.m.)	**Date of report**: 01/09/2009
Shifts: 9	**Local contact(s)**: Jon Ander Gallastegui	*Received at ESRF:*

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Introduction

The main objective of this experiment is to distinguish between the different selenium (Se) species that can be found in Se enriched foods (functional foods), in particular in Se enriched wheat flour. This enrichment can be achieved by two ways: the artificially addition of inorganic Se in wheat cropland or the natural assimilation of Se from the soil. This information will be of key importance to understand the role of Se on the observed health benefits of these foods beyond basic nutrition.[1]

In addition, we are working on a target system that includes a quantitative speciation analysis of Se and their distribution through different wheat tissues after a hydroponic culture with a selenite or selenate biofortification process as well as the monitorization of the consequent bioassimilation process. The aim of this study concerns with a strategy to determine the best practice to anthropogenically enrich wheat. In this way, our first goal is to ascertain the distribution of different Se species that can be found in Se enriched wheat, in particular in roots and shoots to through light on the mentioned bioassimilation process of Se.

The Se speciation information in wheat and also in wheat flour will be of key importance to understand the role of Se on the observed health benefits of functional foods beyond basic nutrition. Nowadays it is well-recognized that the particular physico-chemical form in which an element is present in a sample will determine the toxicity, the biological activity, the bioavailability and the environmental impact of the element. For that reason the topic "speciation" has raised an unusual interest in the last years in areas so diverse such as toxicology, nutrition, agricultural, medical, biochemical and environmental sciences.[2] We applied the synchrotron radiation techniques to the speciation of Se in two kind of samples: enriched wheat plants and wheat flour.

Experimental

XANES analyses were performed at beamline BM25. Details about beamline set-up are given in Table 1. The photon absorption of Se was recorded at the edge energy for its K line at 12658eV, and its $K\alpha_1$ 11224eV and $K\alpha_2$ 12497eV fluorescent line intensities were measured in fluorescence mode. The selection of the detection mode depends upon the sample concentration and the matrix background.[3] Therefore, pure reference compounds were analysed in transmittance mode, while fluorescence detection mode was used for the analysis of unknown samples.

Source	Bending magnet - 16 bunch mode
Source energy	6 GeV
Maximum current	90 mA
Monochromator crystals	Si (1,1,1)
Resolution	$\Delta E/E = 10^{-4}$
Photon flow	$\approx 10^{10}$ - 10^{11} phot/s
Spot size at the sample	1.5 × 1mm
Detectors	3 ionisation chambers (Transmittance) Si (Li) 13 elements (Fluorescence)
Temperature	Room

Table 1. Experimental setup in beamline BM25.

Speciation data were obtained by comparing the spectra from pure compounds, including Na_2SeO_3 and Na_2SeO_4 as inorganic Se compounds, whilst SelenoMethyl-SelenoCysteine (SeMeSeCys) and Selenomethionine (SeMet) were chosen as organic selenoaminoacids. XANES spectra were processed by using Athena data analysis software package.[4] Additionally and in order to observe a possible matrix effect, we analyzed two additional samples of the reference compound SeMet, one diluted in polyethylene and the second one diluted in a wheat flour without Se enrichment.

The wheat hydroponic culture consisted on the next steps: seeds germination, transference of seeds to 1L vessels containing 0.5 strength Hoagland's nutrient solution, growth during one week and exposition to 5 days-long different Se enrichment treatments. These treatments were the following:

- 10µM Na_2SeO_3
- 10µM Na_2SeO_4
- 10µM of mixture (5µM Na_2SeO_3 and 5µM Na_2SeO_4).

Then, plants were harvested, washed with water, divided into roots and shoots and liophylized. Finally, the corresponding samples were homogenised in a mortar and converted into pellets by hydraulic pressure to be analysed at the experimental station of the synchrotron facility. The same sample treatment was done for the wheat flour samples A and B.

Results

Results obtained for the analysis of pure reference Se compounds are presented in Figure 1. As expected, a shift position of the edge features to higher energies as Se becomes more oxidized. It is noteworthy, as seen in Figure 2, that no matrix effect is observed, i.e, both spectra, SeMet diluted in polyethylene and in a blank wheat flour, have the same shape. Nowadays, we have the qualitative results shown in Figure 3 for the samples (enriched wheat shoots and roots and wheat flour A and B).

A qualitative view of the sample spectra obtained shows that for 10µM Na_2SeO_3 and 10µM Na_2SeO_4 treatments Se composition of roots and shoots of each treatment is the same, in the first case the edge is shifted to lower oxidation states as expected because the enrichment is with Na_2SeO_3. However, for the mixture treatment the spectra, and therefore the speciation, of roots and shoots are different, probably due to a different bioassimilation process in comparison with enrichments Na_2SeO_3 or Na_2SeO_4 separately. Finally, the spectra of wheat flours A and B are similar as expected and shifted to the organic Se reference compounds (Figure 3).

For the quantitative analysis, a linear combination fit was carried out using the software package Athena data analysis, obtaining a poor fit that reveals the need for additional reference compounds to be

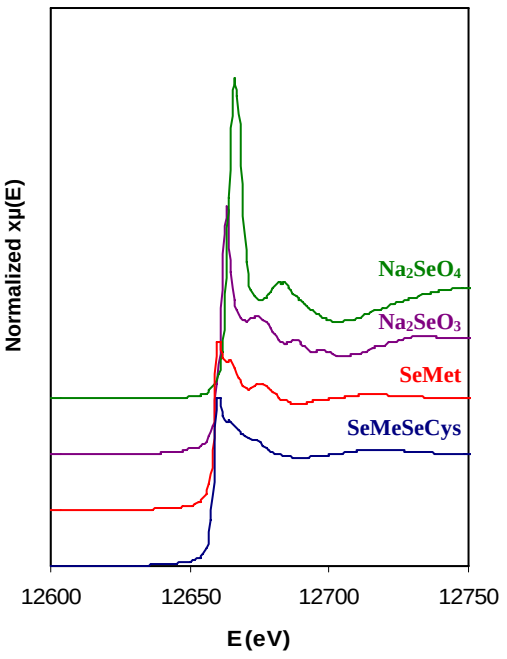

Figure 1. Se K-edge XANES spectra for Se pure reference compounds.

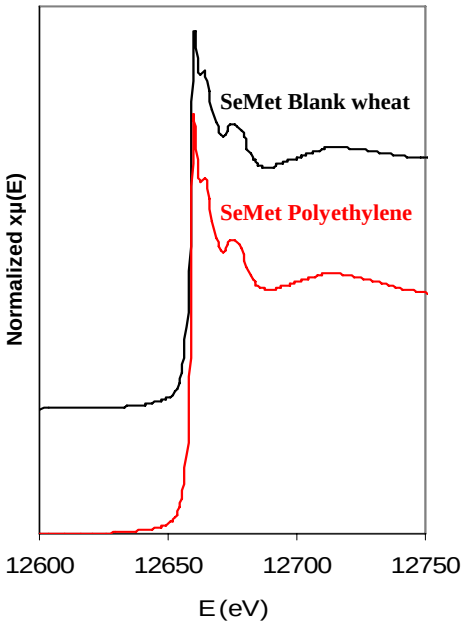

Figure 2. Se K-edge XANES spectra for SeMet diluted with polyethylene and in a blank wheat flour.

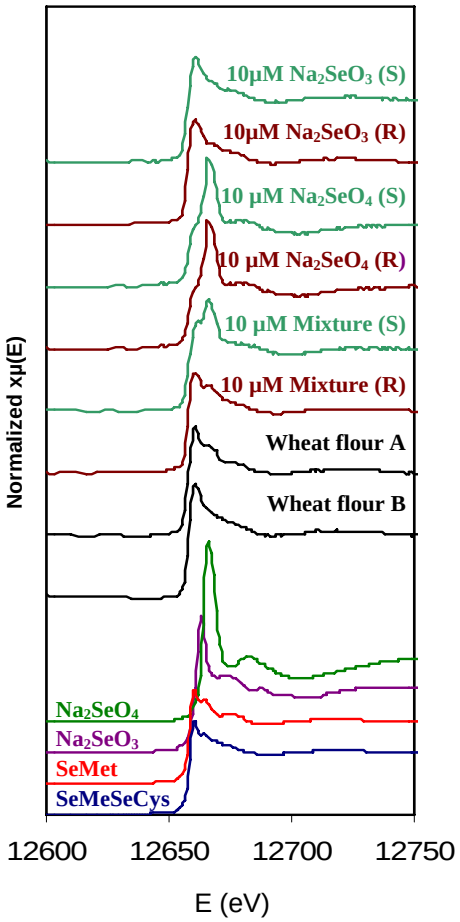

Figure 3. Se K-edge XANES spectra for reference compounds, wheat flour and wheat roots (R) and shoots (S) samples for each Se treatment.

considered in this analysis. Another possible inconvenience is the low total Se concentration. Previous results showed that total Se concentration, determined by ICP-MS after microwave acid digestion, is 10ppm for the wheat flour samples and ranges from 1 to 100ppm in the wheat samples depending on the part of the plant and the Se enrichment treatment. This is the lowest concentration detected for Se in BM25 with its Si (Li) 13 elements detector.

Conclusions and perspectives

As it was shown, the sensitivity of the beamline BM25 allowed us to analyse relatively low Se levels. In addition, it was observed the absence of matrix effect on Se absorption. The obtained spectra of samples showed qualitative differences between the different parts of the plant and the different Se enrichment treatments. However, quantitative analysis of data is difficult at this concentration level and nowadays we are still working on data treatment with a new program, XANES dactyloscope.[5] We are using the PCA algorithm to determine the minimum number and type of probable components to then quantify Se speciation. In order to enrich this study, we need in the future to analyse more reference compounds to perform more appropriate linear combination fitting of reference spectra and to better understand the influence of plants in Se speciation modification, specially to determine quantitatively the Se speciation in our samples.

References

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