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| ESRF | **Experiment title:**<br>The effect of cacao fermentation on the speciation of Cd in cacao bean tissues: the role of phytate | **Experiment number**:<br>EV-468 |
| **Beamline**:<br>BM30 | **Date of experiment**:<br>from: 20 April 2022 to: 25 April 2022 | **Date of report**:<br>8/9/2022 |
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**Report:**

**Context**

Earlier studies revealed that cadmium (Cd) concentrations in cacao nibs can decrease by a factor up to 1.3 during fermentation. It was hypothesized that Cd mobilization during fermentation is related to breakdown of phytate in the nibs and the consecutive release of phytate-bound Cd (Vanderschueren et al., 2023). The objective of this experiment was to identify with bulk XANES the Cd speciation in the cacao beans (i.e. in the nib (seed) and testa (seed envelope)) before and after fermentation, to identify whether Cd is indeed released from phytate during fermentation.

**Experimental setup**

The experiment was performed in 7/8 filling mode (current of 200 mA), with a beam size of 0.23 mm horizontaland 1 mm vertical. The monochromator was a Si(220) crystal. We used a newly installed He cryostat, with sample temparature between 17 and 20 °K. Compared to the previous crytostat, the sample change is easier and the cooling much faster. The detector was a 13-element Ge detector, and a Cd foil was recorded simultaneously in transmission in position 2 for energy calibration.

We recorded the spectra for the unfermented beans, and beans incubated with acetic acid (20 g L-1), at two temperatures (45 and 65 °C). We ran the nib and testa for each condition, and for each sample we ran an experimental duplicate. We also ran some reference spectra to complement our database. After energy calibration and normalization, spectra were treated by linear combination fits using the whole database of Cd references.

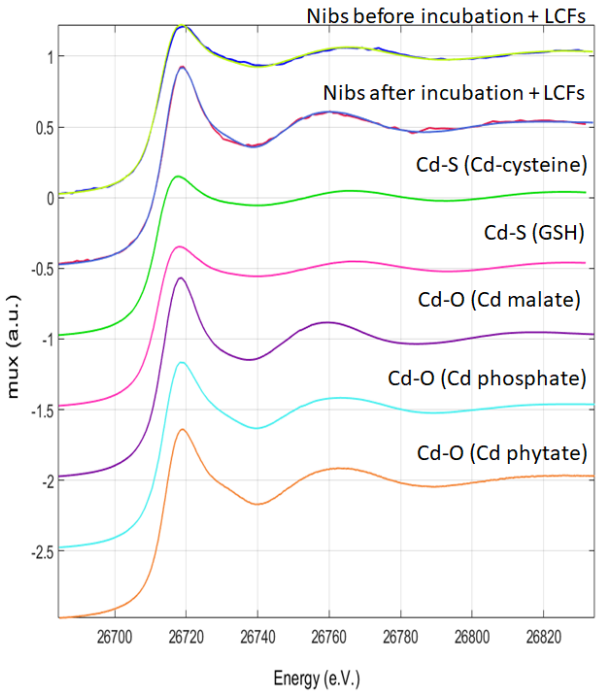


Figure 1: Example of spectra obtained for the beans before and after incubation at 65°C with 20 mg/L acetic acid, compared with representative reference spectra. Linear combinaiton fits evidence a change in Cd speciation during incubation, with a shift from S ligands to O ligands.

Contrary to what was expected, Cd in beans before incubation was not bound to polyphosphate ligands (i.e. phytate) but to thiol ligands. This is probably due to the high Cd concent of these beans collected in the field (around 8 mg $kg^{-1}$ Cd). At this high concentration, a detoxifocation mechanisms seems to be triggered, so that S ligands become the major ligands. In the testa, differences were smaller, but the spectra before and after incubation also differed. When cacao beans are incubated at high temperature and in the presence of acetic acid, this Cd-S complex is dissociated, as expected from complexation constants, and Cd binds to weaker ligands, which explains its enhanced mobility. This knowledge is important for the development of postharvest mitigation strategies for Cd in cacao.

An article presenting the results is in preparation.