

# Characterization of Sulfur Compounds in Coffee Beans by Sulfur K-XANES Spectroscopy

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**Abstract.** In this ‘feasibility study’ the influence of roasting on the sulfur speciation in Mexican coffee beans was investigated by sulfur K-XANES spectroscopy. Spectra of green and slightly roasted beans could be fitted to a linear combination of ‘standard’ reference spectra for biological samples, whereas longer roasting obviously involves formation of additional sulfur compounds in considerable amounts.

**Keywords:** XANES spectroscopy, coffee beans, roasting

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## INTRODUCTION

Besides sulfur in the coffee proteins resulting from the amino acids cysteine and methionine, several (mainly volatile) sulfur containing compounds are present in coffee that play an important role for the aroma and flavor [1,2]. It was the purpose of this “explorative” study to analyze the influence of roasting on sulfur speciation in coffee beans by using X-ray absorption near edge structure (XANES) spectroscopy at the sulfur K-edge. In comparison with other techniques, XANES measurements can be carried out ‘in situ’, i.e. no (wet chemical) extraction processes are required.

## EXPERIMENT

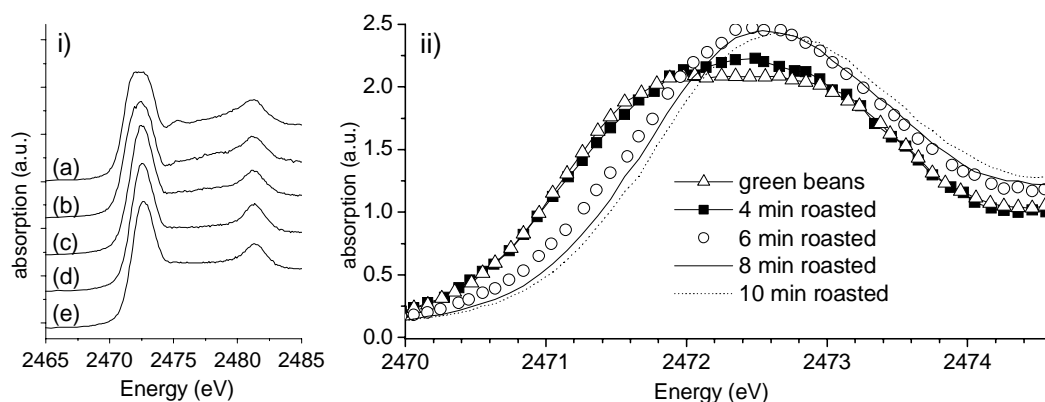
### Samples and Sample Preparation

Green Mexican coffee beans were prepared in a test roaster (different durations of roasting: 4, 6, 8, 10 min, temperature approx. 190 °C). In order to include also effects of drastic overroasting, the maximum duration of roasting (10 min) was extended beyond the point where one would normally stop, producing pitch black beans (by far too dark for the preparation of a coffee beverage). After roasting, coffee beans were vacuum sealed (plastic bags) and kept in a refrigerator.

Shortly before the measurements, coffee beans were ground in a coffee grinder with a millwork/grinding gear under ambient atmospheric conditions. Powder samples thus obtained were uniformly spread on adhesive sulfur free kapton tape and covered with polypropylene foil.

### XANES-measurements

XANES (X-ray absorption near edge structure) spectra at the sulfur K-edge were recorded at the Center for Advanced Microstructures and Devices (CAMD, Louisiana State University) using bending magnet radiation at the DCM beamline [3]. The storage ring was operated at an energy of 1.3 GeV with electron currents between 200 and 80 mA. The X-ray beam was monochromatized with a modified Lemonnier type double crystal X-ray monochromator equipped with InSb(111)-crystals [4]. Measurements were performed in fluorescence mode. The incident monochromatic x-ray intensity ( $I_0$ ) was measured with a 10 cm-long ionization chamber (60 mbar air). The fluorescence intensity  $I_F$  was measured with a (cryogenic) multi-element Germanium detector (8 active elements) positioned perpendicular to the beam direction. The sample was oriented at 45° to the incident beam. Sample chamber and ionization chamber were flushed with helium to minimize the



**FIGURE 1.** Sulfur K-edge XANES spectra of Mexican coffee beans: (a) green beans (b) roasted for 4 min, (c) roasted for 6 min, (d) roasted for 8 min, (e) roasted for 10 min.

attenuation of the fluorescence radiation. For energy calibration of the spectra, a XANES spectrum of  $\text{ZnSO}_4$  was recorded in transmission mode (transmitted intensity measured with another 10 cm-long ionization chamber behind the sample) and the maximum of the first resonance (“white line”) set to an energy of 2481.44 eV. According to the step width, this value is reproducible to  $\pm 0.1$  eV. Spectra were scanned with 0.5 eV steps in the pre-edge (2440–2468 eV), with 0.1 eV steps in the near edge (2468–2485 eV) region, and from 2485–2520 eV (post edge) with 0.3 eV steps. Integration times were 1 s (calibration in transmission mode) and 2 s (fluorescence mode) per point.

A linear background determined in the pre-edge region was subtracted from the raw data (ratio of  $I_F$  and  $I_0$ ) to correct the absorption from higher shells and from supporting materials. The spectra were normalized at 2510 eV, where the variation of the absorption cross-section is already very small. Samples were measured twice and resulted spectra were averaged.

## RESULTS AND DISCUSSION

Several differences between XANES spectra of coffee beans of varying degrees of roast are shown in Figure 1. The spectrum of the green beans (Fig. 1i), (a) shows a first strong resonance with a maximum at approx. 2472.4 eV (“white line”), most probably resulting from a superposition of C-S and S-H (cysteine, methionine, glutathione) and disulfide (oxidized glutathione) bonds, which is the typical environment of sulfur in biological systems, e.g. [5]. With increasing roasting time, the white line is slightly

increasing in intensity and shifting towards higher energies, and its width is decreasing (Fig. 1 ii), which can be assigned to an oxidation (and thus a decrease) of S-S bonds; similar changes in the white line structure have been observed for the system “wheat gluten proteins” [6,7]. Besides the white line peak, all spectra in Figure 1 show a more or less pronounced structure at approx. 2481.4 eV, being evidence of the presence of a sulfate. Presence of sulfate in coffee beans – although not mentioned explicitly in the literature – is not surprising as sulfate is present within the majority of plants, e.g. [8]. The sulfate concentration in the beans is not significantly affected by the roasting process. In the spectra of green beans a weak maximum at 2475.3 eV can be observed. With increasing duration of roast, this spectral feature, which might be assigned to a Rydberg-state indicating the presence of S-H bonds [9] or the presence of a sulfoxide [7], disappears. Whether the disappearance of this structure might be correlated to a “thermal oxidation” or “thermal ageing” of sulfur during the roasting process will be elucidated in further experiments.

### Quantitative Analysis / Fitting Attempts

The vast majority of sulfurous molecules in green coffee beans are considered to be proteins [2,10]. A multitude of different sulfur-containing aroma compounds in coffee, for the most part formed during the roasting process, have been identified [1,2]—however, they occur in comparatively low concentrations [2]. Therefore it should be feasible to fit at least the green bean XANES spectra to a linear combination of suitable reference spectra using a least

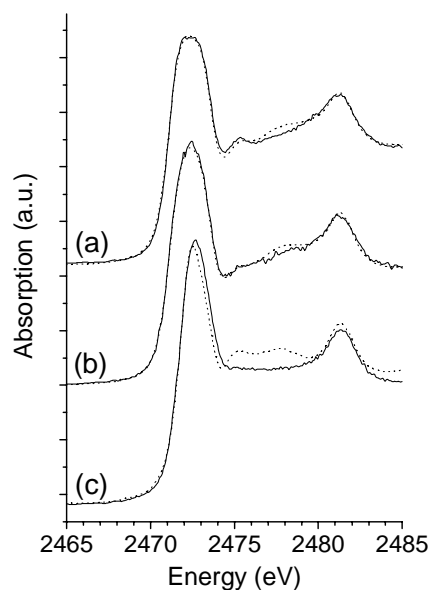
square fitting routine. In this case, reduced glutathione (GSH), oxidized glutathione (GSSG), methionine, dimethyl-sulfoxide (DMSO), cysteic acid and zinc sulfate were used as reference compounds. In previous studies, e.g. [7], this set of reference compounds has proved to be suitable for fitting XANES spectra of biological samples. Spectra of *cyclo*-octasulfur, polymeric sulfur, cysteine and cystine were also included in the fitting attempts, but were discarded by the fitting program. For a quantitative analysis, the fitting and plotting package WinXAS, a least square fitting routine, was used [11,12]. Additional details concerning the quantitative analysis of the XANES spectra approach, especially its verification, potential, and restrictions have been published elsewhere [13]. The errors of the percentage contributions of sulfur species can be estimated to be smaller than  $\pm 10\%$  (absolute value) [13].

For the green and slightly roasted Mexican beans (Fig. 2 (a), (b)) the fits are -for biological samples- in relatively good agreement with the measured spectra. The fitting procedure yielded the following results for green beans (Fig. 2 (a)): 18% GSH, 57% GSSG, 16% methionine, 1% DMSO, 3% cysteic acid and 5% sulfate. For slightly roasted beans, similar results (within the margin of error) were obtained, whereas for higher degrees of roast a massive discrepancy between measured spectra and corresponding WinXAS fits occurs (Fig. 2 (c), representative example for fitting higher degrees of roast).

The experimental results suggest that, in accordance with the literature [2,6,7,10], the vast majority of sulfur atoms in green and slightly roasted coffee beans are part of the protein matrix (acceptable quality of fit), whereas in the course of the roasting process thermal degradation of proteins and reactions of proteins (protein fragments) with other coffee compounds (esp. carbohydrates) (and perhaps with "air" during the grinding process) produce considerable amounts of additional sulfur compounds (decreasing quality of fits, white line shift). Identification of those substances (esp. cyclic thiols, e.g. furfurylthiol [1]) has not yet been achieved in this spectroscopy study, but is an objective of planned future investigations.

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**FIGURE 2.** Sulfur K-edge XANES spectra of green (a), slightly (4 min, (b)) roasted and overroasted (10 min, (c)) Mexican coffee beans (solid) and corresponding WinXAS fits (dotted).

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